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Bioefficacy of Sulfur-Oxidizing Bacteria and Sulfur Concentrations in Enhancing Onion (*Allium cepa* L.) Growth Parameters

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TWO ISOLATES of sulfur oxidizing bacteria (SOB) were isolated from the rhizosphere of onion plants (*Allium cepa* L.), Assiut Governorate, Egypt. These two isolates SOB12 and SOB15 were identified using 16S rRNA gene sequencing as *Bacillus spizizenii* strain B566 and *Priestia aryabhattai* strain B567, respectively. Aimed to evaluate the effect of two sulfur-oxidizing bacterial in combination with different sulfur concentrations, on the growth, nutrient uptake, and biochemical composition of onion (*Allium cepa* L.) plants. Thirteen treatments were applied in pot experiments using sulfur doses at 50%, 100%, and 150% of the recommended rate, either alone or combined with bacterial inoculants. Both bacterial strains positively influenced plant performance, with SOB15 generally outperforming SOB12 and sulfur alone across most measured parameters. However, the best overall results were observed in treatments combining SOB12 and SOB15 individually or in combination with 50% of the recommended sulfur dose. These treatments significantly enhanced plant height, leaf number, bulb weight, photosynthetic pigments, nutrient uptake (N, P, K, S), and biochemical constituents such as carbohydrates, amino acids, phenolics, and pyruvic acid. Notably, at 50% sulfur, co-inoculation (SOB12+SOB15) significantly increased bulb dry weight by 127% relative to 50% sulfur without inoculation; at 100% sulfur, SOB15 increased nitrogen uptake by 45% and bulb fresh weight by 65% versus sulfur alone; and at 150% sulfur, SOB15 raised total carbohydrate concentration by 146% compared with sulfur alone. These findings highlight the potential of SOB12 and SOB15, particularly *P. aryabhattai* (SOB15), as effective biofertilizers for promoting sustainable onion cultivation under sulfur-limited conditions.

Keywords: Sulfur; Nutrient uptake; Plant growth; Biochemical constituents.

1. Introduction

Sulfur is a vital macronutrient required for the successful cultivation of onion (*Allium cepa*), playing a key role in plant growth, development, and productivity. It is a fundamental constituent of sulfur containing amino acids such as cysteine and methionine, which are essential for protein synthesis in plants (Zenda et al., 2021; Shah et al., 2022). In addition, sulfur contributes to the formation of volatile sulfur compounds that define the unique flavor, aroma, and pungency of onions, while also enhancing plant resilience against diseases and environmental stresses (Luo et al., 2024; Ma et al., 2024). Onions primarily absorb sulfur in the form of sulfate (SO_4^{2-}) from the soil, making it critical to maintain adequate sulfur levels in agricultural systems (El-Bakry et al., 2024). Sulfur deficiency not only results in chlorosis, stunted growth, and reduced bulb quality, but also diminishes the characteristic pungency of onions, thereby reducing their market value (Yebalework et al., 2024). To overcome such limitations, sulfur-rich fertilizers and microbial amendments particularly sulfur-oxidizing bacteria have been explored to improve sulfur bioavailability in soil and enhance crop performance.

Onion is one of the most widely cultivated vegetable crops globally, valued for its culinary versatility, nutritional richness, and economic importance (Ochar & Kim, 2023). It is a notable source of vitamins and antioxidants, including vitamin C and quercetin, which contribute to immune system support and human health (Muscolo et al., 2025). The crop also benefits from excellent post-harvest storage capacity, facilitating large-scale distribution and export. Optimal onion growth requires fertile, well-drained soils and a balanced supply of key macronutrients, particularly nitrogen (N), phosphorus (P), potassium (K), and sulfur (S) (Majid et al., 2024). However, modern onion cultivation is increasingly challenged by soil nutrient depletion, pest infestations, and fungal diseases such as downy mildew and white rot. Abiotic factors, including drought and temperature extremes, further compromise crop productivity (Elenany et al., 2024). These challenges underscore the need for sustainable and innovative agricultural practices among which the application of beneficial microbes such as

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sulfur-oxidizing bacteria (SOB) is gaining traction as a means to promote plant nutrition, soil health, and environmental safety.

Sulfur-oxidizing bacteria are a specialized group of microorganisms involved in the biological sulfur cycle. They convert various reduced sulfur compounds such as hydrogen sulfide (H_2S), elemental sulfur (S^0), and sulfur dioxide (SO_2) into sulfate (SO_4^{2-}), the plant-available form of sulfur (Ranadev et al., 2023; Yim, 2019). SOB enhance plant growth primarily by accelerating the transformation of these reduced sulfur compounds into sulfate through enzymatic systems such as sulfur oxidase and sulfite oxidase, which facilitate electron transfer during sulfur oxidation. In addition, many SOB release organic acids that lower rhizospheric pH, thereby increasing the solubility of phosphorus, potassium, and micronutrients. SOB also stimulate microbial diversity and enzymatic activity in the rhizosphere, improving soil health and root nutrient uptake. Collectively, these processes not only increase sulfur bioavailability but also enhance plant vigor and resilience under sulfur-limited conditions (Mao et al., 2023; Ranadev et al., 2023; Vishnu et al., 2024). Species such as *Thiobacillus* are well known for their dual ability to oxidize sulfur and solubilize phosphorus, iron, and zinc functions that are particularly beneficial in nutrient-depleted or alkaline soils (Rana et al., 2020; Vishnu et al., 2024). While SOB naturally occur in various environments, their populations are often limited in agricultural soils, making microbial inoculation a promising intervention. Indeed, the inoculation of *Thiobacillus* sp. with elemental sulfur has been reported to significantly improve plant growth parameters and crop yield (Vidyalakshmi et al., 2009). Furthermore, the co-inoculation of SOB and nitrogen-fixing bacteria such as *Azotobacter chroococcum* has shown synergistic effects, markedly increasing onion plant height, yield (by 221%), and nitrogen uptake (by 629%) in sulfur-deficient soils (Awad et al., 2011). In addition to improving nutrient uptake, sulfur and sulfur-oxidizing bacteria have also been shown to enhance biochemical quality parameters of onion bulbs, including protein content, phenolic compound accumulation, and pyruvic acid levels, which are directly linked to nutritional value, antioxidant capacity, and pungency (Dalamu et al., 2010; Zenda et al., 2021; Sagar et al., 2022).

This study aims to evaluate the effects of varying sulfur concentrations and inoculation with beneficial sulfur-oxidizing bacteria on the growth performance, nutrient uptake (N, P, K, S), and biochemical composition including photosynthetic pigments, protein, carbohydrate, amino acid, phenolic content, and pyruvic acid of onion (*Allium cepa* L.) plants. The goal is to determine optimal treatment combinations that promote sustainable and high-quality onion production under field-relevant conditions.

2. Materials and Methods

2.1. Bacterial inoculum preparation and application

Two sulfur-oxidizing bacterial strains were used in this study: SOB12 (*Bacillus spizizenii* strain B566) and SOB15 (*Priestia aryabhattai* strain B567), deposited in the culture collection of the Assiut University Mycological Center (AUMC). Each strain was cultured separately in nutrient broth and incubated at 30 °C for 48 hours under continuous shaking (150 rpm). The bacterial cells were harvested and adjusted to a concentration of approximately 10^8 CFU/mL using sterile distilled water. For inoculation, 50 mL of the bacterial suspension was applied to the soil around the root zone of each plant at the time of transplanting. In treatments where both strains were applied (SOB12 & SOB15), equal volumes (25 mL each) of both suspensions were mixed and applied together. Inoculation was done only once at the time of transplanting. Control treatments received the same volume of sterile nutrient broth without bacteria.

2.2. Plant material, fertilizers, and experimental design

Sixty-day-old seedlings of onion **(*Allium cepa* L., cv. Giza 6)** were obtained from the Department of Crops, Assiut University Farm (AUF). The pot experiment was conducted under open-field conditions at AUF during November, 2024. Average daytime temperature ranged between 22–28 °C with approximately 10.5 h of natural daylight. Relative humidity in Assiut during this period is typically moderate (45–60%), according to regional meteorological data. Irrigation was managed to maintain soil moisture near field capacity. Uniform, healthy seedlings were selected for transplanting. Before planting, a composite soil sample was collected from the experimental site at a depth of 0–30 cm. The soil was air-dried, sieved through a 2 mm mesh, and its physicochemical properties were determined according to Jackson (1973) in Table 1. The soil was then mixed with sand in a 2:1 ratio (w/w), and 7 kg of the mixture was filled into each plastic pot (20 cm height × 26 cm diameter). The experiment included 13 treatments (Table 2), representing various combinations of agricultural sulfur concentrations and microbial inoculation strategies. Agricultural sulfur was applied at three concentrations: 50%, 100%, and 150% of the recommended dose. Two sulfur-oxidizing bacterial strains were used as inoculants: (SOB12) and (SOB15).

In some treatments, both strains were applied together as a mixed inoculum. The bacterial inoculants were applied at the time of transplanting by soil drenching around the root zone. All treatments received the

recommended doses of mineral fertilizers: urea (46% N) as a nitrogen source, potassium sulfate (50% K_2O) as a potassium source, and single superphosphate as a phosphorus source. Fertilizers were mixed with the soil prior to transplanting. Each treatment was replicated five times, resulting in a total of 65 pots arranged in a completely randomized design (CRD), as demonstrated in the experimental flowchart (Fig 1). For statistical analysis, the 13 treatments were classified into four main groups based on the type of microbial inoculation and the level of sulfur application.

Table 1. Physico-chemical properties of experimental soil before planting.

Soil Properties	Values	Soil Properties	Values
Particle size distribution		**Soluble cations (mmol/L)**	
Clay, %	20.3	Ca^{++}	3
Silt, %	23.5	Mg^{++}	2.4
Fine Sand, %	30.2	Na^{+}	8.1
Coarse Sand %	26	K^{+}	0.5
Texture grade	Sandy Loam	**Soluble anions (mmol/L)**	
		HCO_3^-	0.3
		Cl^-	11.7
		$SO_4^=$	2
pH (1:2.5 soil water suspension)	7.76	**Available macronutrients (mg kg^{-1} soil)**	
EC (dS m^{-1})	1.4	N	22.3
Field capacity	28.6	P	1.25
Wilting point (%)	12.3	K	135
Bulk density (g cm^{-3})	1.44	S	0.67

2.3. Growth and yield measurements

At harvest (after 3 months), several growth and yield parameters were recorded to evaluate the response of onion plants to the applied treatments. These included plant height (cm), number of leaves per plant, fresh weight (g), dry weight (g) of the whole plant and length of bulbs & necks per plant. The diameter of onion bulbs (cm) and necks were also measured using a caliper measuring tool to assess bulb development. Dry weight was determined by oven-drying the plant samples at 65 °C for 48h. All measurements were performed on **five** replicates per treatment, and the data were statistically analyzed to assess treatment effects.

2.4. Determination of Microbial Activity in Soil

Microbial activity in soil was determined using fluorescein diacetate (FDA) hydrolysis following the method of Adam and Duncan (2001). Briefly, 2 g of soil were incubated with 20 ml of 60 mM sodium phosphate buffer (pH 7.6) and 100 µl of FDA solution at 25°C with shaking. After 45 minutes, the reaction was stopped with 2 ml of acetone, and the mixture was filtered. Absorbance of the filtrate was measured at 490 nm using a spectrophotometer. A standard curve was generated using serial dilutions of fluorescein (0–50 µg/ml) to calculate the amount of fluorescein released as an indicator of microbial activity.

2.5. Photosynthetic pigment analysis

Photosynthetic pigments, including chlorophyll a, chlorophyll b, and carotenoids, were extracted from fresh leaf samples using 95% ethanol and quantified spectrophotometrically according to the method of Lichtenthaler (1987). Pigment extraction was performed at 60 °C until the tissues became colorless, and the absorbance of the extracts was measured at 663, 644, and 452 nm using a Unico UV-2100 spectrophotometer. Pigment concentrations were calculated and expressed as mg/g fresh weight (FW).

2.6. Macronutrient analysis in onion bulbs

Dried onion bulb samples (0.5 g) were accurately weighed and subjected to wet acid digestion using a mixture of sulfuric acid (H_2SO_4) and perchloric acid ($HClO_4$) according to the method of Pratt and Chapman (1961). The resulting digests were used for the determination of total nitrogen, phosphorus, potassium, and sulfur contents. Total nitrogen was measured using the micro-Kjeldahl method (Jackson, 1973), while total phosphorus was determined colorimetrically using the stannous chloride phosphomolybdic acid method in a sulfuric acid medium (Jackson, 1973). Total potassium was estimated by flame photometry (Jackson, 1973). Total sulfur content was assessed using the modified barium sulfate precipitation method, in which all sulfur species (organic and inorganic) were converted to sulfate and quantified turbidimetrically using barium chloride ($BaCl_2$), as described by Senthilkumar et al. (2020).

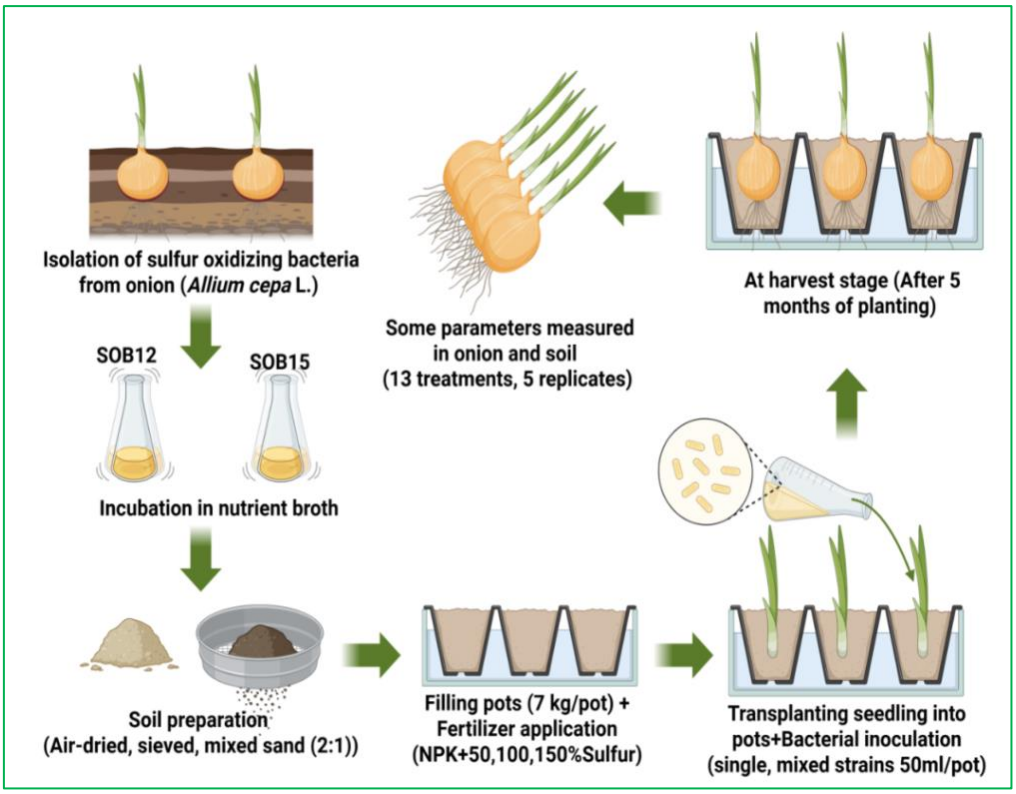

Fig. 1. Flowchart illustrating the experimental procedure using BioRender (version 2025) for evaluating the effect of sulfur-oxidizing bacteria (SOB) and elemental sulfur on onion plants (Allium cepa L.) growth. The steps include bacterial isolation, inoculum preparation, soil analysis and amendment, seedling transplantation, fertilizer application, microbial inoculation, as well as harvesting and conducting physiological and biochemical measurements on both onion plants and soil. Each treatment was replicated five times, resulting in a total of 65 pots arranged in a completely randomized design (CRD).

2.7. Biochemical constituents

The biochemical composition of onion bulbs was assessed through several standard assays. Pyruvic acid content was estimated according to the method of Anthon and Barret (2002). Fresh onion bulb extract was reacted with 2,4-dinitrophenylhydrazine (DNPH), and the resulting hydrazone complex was measured spectrophotometrically at 520 nm. Pyruvic acid concentrations were then calculated and expressed as µmol/g fresh weight (FW). Total phenolic content was determined using the Folin–Ciocalteu method described by Singleton and Rossi (1965). Ethanol extracts of fresh tissue were reacted with Folin reagent and sodium carbonate, and the absorbance was measured at 765 nm. Gallic acid served as the standard, and the results were expressed as mg gallic acid equivalent (GAE)/g FW. For total soluble carbohydrates, the phenol–sulfuric acid colorimetric assay of Dubois et al. (1956) was employed. A known weight of fresh tissue was extracted in ethanol, and absorbance was recorded at 490 nm using glucose as the standard. The carbohydrate content was expressed as mg/g FW. Free amino acids were quantified using the ninhydrin-based colorimetric method of Lee and Takahashi (1966). Fresh tissue extracts were reacted with ninhydrin reagent, and absorbance was measured at 570 nm. Glycine was used as the standard, and values were expressed as µmol/g FW. Finally, total soluble protein was calculated following the procedure of Hymowitz et al. (1972), in which the nitrogen percentage was determined and multiplied by 6.25 to estimate the protein concentration of onion tissues.

2.8. Statistical analysis

Based on the experimental layout, the 13 treatments (Table 2) were categorized into **four** distinct groups according to the type of sulfur levels and microbial inoculation. These groups were: **(A)** mineral fertilization, levels of sulfur without bacterial inoculation, **(B)** 50% sulfur across inoculation bacterial types, **(C)** 100% sulfur across inoculation bacterial types, and **(D)** 150% sulfur across inoculation bacterial types. one-way ANOVA followed by Tukey's test was performed separately for each group, Each treatment was replicated five times and values are presented as mean ± standard deviation (SD) using GraphPad Prism software (v10.4.0). Significant differences were determined at $p \leq 0.05$.

Table 2. Description of the 13 experimental treatments combining NPK, different sulfur levels and sulfur-oxidizing bacterial inoculations applied to onion (*Allium cepa* L.) plants.

Treatments	Inoculation	Treatments	Inoculation
T_1	Control (NPK only)	T_8	NPK+50% Sulfur +(SOB15)
T_2	NPK+50% Sulfur	T_9	NPK+100% Sulfur +(SOB15)
T_3	NPK+100% Sulfur	T_{10}	NPK+150% Sulfur +(SOB15)
T_4	NPK+150% Sulfur	T_{11}	NPK+50% Sulfur +(12&15 SOB)
T_5	NPK+50% Sulfur +(SOB12)	T_{12}	NPK+100% Sulfur +(12&15 SOB)
T_6	NPK+100% Sulfur +(SOB12)	T_{13}	NPK+150% Sulfur +(12&15 SOB)
T_7	NPK+150% Sulfur +(SOB12)		

3. Results

3.1. Physico-chemical properties of the experimental soil

The physico-chemical analysis of the experimental soil before planting is presented in Table 1. The soil texture was classified as sandy loam, composed of 20.3% clay, 23.5% silt, 30.2% smooth sand, and 26.0% rough sand. The soil had a neutral to slightly alkaline pH of 7.76 and an electrical conductivity (EC) of 1.4 dS/m, indicating non-saline conditions suitable for onion growth. Soluble cations in the soil solution were predominantly Na^+ (8.1 meq/L), followed by Ca^{2+} (3.0 meq/L), Mg^{2+} (2.4 meq/L), and K^+ (0.5 meq/L). Regarding the anionic composition, Cl^- (11.7 meq/L) was the dominant ion, followed by SO_4^{2-} (2.0 meq/L) and HCO_3^- (0.3 meq/L). The field capacity and wilting point of the soil were recorded as 28.6% and 12.3%, respectively, indicating moderate water retention capacity. The bulk density of the soil was 1.44 g/cm³, suggesting good porosity and aeration, conducive for root development and microbial activity.

3.2. Bacterial inoculum

Two sulfur-oxidizing bacterial strains were used in this experiment: ***Bacillus spizizenii* strain AUMC B566** (referred to as SOB12) and ***Priestia aryabhattai* strain AUMC B567** (referred to as SOB15). These strains were previously isolated and identified through 16S rRNA gene sequencing and deposited at the Assiut University Mycological Center (AUMC). The culture was incubated at 30 ºC for 48 hours prior sending to the molecular Biology Research Unit, Assiut University for DNA extraction. Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea was used. The extracted DNA sample was sent to SolGent Company, Daejeon South Korea for polymerase chain reaction (PCR) and gene sequencing.

3.3. Effect of sulfur-oxidizing bacteria and sulfur on onion biomass production

The results in Table 3 show clear variations in the fresh and dry weights of onion plant parts (leaves, neck, and bulbs) across the different treatment groups. In the control group (A), the application of sulfur significantly enhanced leaf and bulb biomass compared to NPK alone. The treatment with 100% sulfur produced the highest leaf fresh weight (56.86 g plant^{-1}), while 150% sulfur resulted in the greatest bulb fresh (21.88 g plant^{-1}) and dry weight (2.23 g plant^{-1}), indicating a favorable effect of increasing sulfur concentration even in the absence of microbial inoculants.

When 50% sulfur was applied in combination with sulfur-oxidizing bacteria (Group B), a noticeable improvement in bulb dry weight was observed, particularly with the co-inoculation of *Bacillus spizizenii* (SOB12) and *Priestia aryabhattai* (SOB15), which achieved the highest bulb dry weight (4.23 g plant^{-1}). Although most differences in shoot biomass within this group were not statistically significant, the increase in bulb weight highlights the potential of microbial inoculation to enhance sulfur utilization and promote storage organ development (Table 3). Similarly, in Group C, where plants received 100% sulfur along with bacterial inoculants, treatments inoculated with SOB15 alone and the mixed inoculum both led to considerable increases in bulb biomass. The highest bulb fresh weight (27.17 g plant^{-1}) was observed with SOB15, while the dry weight peaked in the mixed inoculum treatment (2.96 g plant^{-1}), with statistically significant differences recorded for dry bulb weight.

Table 3. Effect of sulfur-oxidizing bacterial inoculation and sulfur concentrations on fresh and dry weights (g plant^{-1}) of leaves, neck, and bulb of onion plants at harvest.

Groups	Treatments	Leaves (g plant^{-1})		Neck (g plant^{-1})		Bulb (g plant^{-1})	
		Fresh wt.	Dry wt.	Fresh wt.	Dry wt.	Fresh wt.	Dry wt.
A	T_1	34.12b	3.34a	11.11b	1.19b	11.48b	1.28bc
	T_2	53.75ab	4.75a	22.23a	2.30a	16.82ab	1.86ab
	T_3	56.86a	5.04a	21.25a	1.98a	16.42ab	1.19c
	T_4	50.85ab	4.57a	18.78ab	1.77ab	21.88a	2.23a
	F test	*	NS	*	**	**	***
B	T_2	53.75a	4.75a	22.23a	2.30a	16.82b	1.86b
	T_5	50.60a	4.36a	19.03a	1.60ab	26.53ab	2.63ab
	T_8	43.44a	3.94a	13.78a	1.37b	21.83ab	2.27b
	T_{11}	48.00a	4.04a	19.53a	1.83ab	34.91a	4.23a
	F test	NS	NS	NS	*	*	**
C	T_3	56.86a	5.04a	21.25a	1.98a	16.42b	1.19b
	T_6	52.23a	4.63a	18.18a	1.65a	24.16ab	2.64ab
	T_9	49.98a	4.35a	19.28a	1.93a	27.17a	3.46a
	T_{12}	56.00a	4.77a	22.65a	2.15a	24.47ab	2.96a
	F test	NS	NS	NS	NS	*	**
D	T_4	50.85a	4.57a	18.78a	1.77a	21.88b	2.23a
	T_7	61.78a	5.21a	21.80a	1.95a	31.36ab	3.04a
	T_{10}	62.97a	5.56a	22.98a	2.29a	33.88ab	3.97a
	T_{13}	51.08a	4.13a	20.62a	1.97a	39.35a	3.85a
	F test	NS	NS	NS	NS	*	NS

Values are presented as mean values (n = 5 replicates). Different letters within the same group indicate significant differences at $p \leq 0.05$ according to Tukey's test. **F test**: **NS** = not significant, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. Grouping for one-way ANOVA: Group **A** (no inoculation across sulfur levels), Group **B** (50% sulfur across inoculation types), Group **C** (100% sulfur across inoculation types), Group **D** (150% sulfur across inoculation types).
T_1:Control (NPK only), T_2: NPK+50%S, T_3:NPK+100%S, T_4:NPK+150%S,T_5:NPK+50%S +(SOB12), T_6: NPK+100%S +(SOB12), T_7:NPK+150% S+(SOB12),**T8**:NPK+50% S+(SOB15), T_9: NPK+100% S+(SOB15),T_{10}:NPK+150% S+(SOB15), T_{11}:NPK+50% S+(12&15 SOB), T_{12}: NPK+100%S+(12&15 SOB),T_{13}:NPK+150%S+(12&15 SOB).

In Group D, which combined 150% sulfur with bacterial inoculation, co-inoculated plants again demonstrated superior performance. Although differences among treatments were not statistically significant, the treatment receiving both SOB strains showed the highest bulb fresh weight (39.35 g plant^{-1}), indicating a positive trend toward enhanced productivity with increasing sulfur levels and microbial synergy (Table 3). Taken together, the results indicate that sulfur application enhances onion biomass, particularly bulb development, and this effect is further amplified when combined with sulfur-oxidizing bacteria. Among the four groups, the treatment combining 150% sulfur with dual inoculation of SOB12 and SOB15 (Group D) consistently showed the highest values for both bulb fresh and dry weights. This highlights the superior performance of co-inoculated plants under elevated sulfur levels, suggesting a potential synergistic effect between sulfur supply and microbial biofertilization in maximizing onion productivity.

3.4. Soil microbial activity as influenced by SOB inoculation and sulfur levels

Microbial activity in soil, measured via FDA hydrolysis at harvest, showed no statistically significant differences among treatments ($p > 0.05$), although some notable trends were observed, as illustrated in Fig 2. In the comparison between the control (NPK only: 28.41 µg F./g/h) and treatments with increasing sulfur levels alone 50% sulfur (29.70 µg F./g/h), 100% sulfur (28.08 µg F./g/h), and 150% sulfur (33.19 µg F./g/h) microbial activity remained relatively similar, suggesting that sulfur application alone had limited influence on microbial stimulation.

When 50% sulfur was combined with bacterial inoculation, microbial activity was 28.41 µg F./g/h with SOB12, 34.37 µg F./g/h with SOB15, and 24.09 µg F./g/h with the mixed inoculum (SOB12 + SOB15). Although not statistically significant, these values show a slight enhancement with SOB15 and a reduction when both strains were combined. At the 100% sulfur level, microbial activity increased from 28.08 µg F./g/h with no inoculation to 34.09 µg F./g/h with SOB12, 35.92 µg F./g/h with SOB15, and reached the highest value of 44.67 µg F./g/h when both strains were applied together, indicating a potentially synergistic effect under optimal sulfur conditions.

Under 150% sulfur, microbial activity was 33.19 µg F./g/h without inoculation, 28.34 µg F./g/h with SOB12, 38.95 µg F./g/h with SOB15, and 37.33 µg F./g/h with the dual inoculum. Again, while statistical differences were not detected, the results suggest that SOB15 alone or in combination with SOB12 enhanced microbial activity more than sulfur alone or SOB12 alone.

Overall, despite the absence of statistical significance, microbial activity tended to increase with SOB inoculation particularly when combined with 100% or 150% sulfur levels highlighting a potential beneficial interaction between sulfur fertilization and microbial enhancement, as presented in Fig 2.

3.5. Effect of bioinoculants and sulfur on photosynthetic pigments in onion leaves

Fig. 3 shows that inoculation with sulfur-oxidizing bacteria and sulfur application had a pronounced impact on the accumulation of photosynthetic pigments in onion leaves. Chlorophyll a content exhibited significant variation among treatments, with the highest level recorded in plants treated with 50% sulfur combined with *Bacillus spizizenii* (SOB12) (0.63 mg g^{-1} FW). This was followed by the co-inoculation treatment (SOB12&15) and the 100% sulfur treatment, whereas the control group exhibited the lowest value (0.32 mg g^{-1} FW). These differences were statistically significant in all treatment groups except under 100% sulfur conditions. Chlorophyll b was less responsive, with the highest concentration (0.10 mg g^{-1} FW) also observed under the 50% S + SOB12 treatment. However, no significant differences were found in most treatment groups, except for Group B (50% sulfur), indicating limited sensitivity of chlorophyll b to the experimental conditions. Carotenoid content followed a similar trend to chlorophyll a, with significant increases in response to microbial inoculation under low sulfur input. The 50% S + SOB12 treatment led to the highest carotenoid level (0.26 mg g^{-1} FW), followed by the co-inoculated group. The control treatment showed the lowest carotenoid accumulation (0.15 mg g^{-1} FW), and significant treatment effects were detected only in Group B. Total photosynthetic pigment content was significantly enhanced by the combined use of SOBs and sulfur fertilization, particularly in the 50% S + SOB12 treatment (0.99 mg g^{-1} FW). Although other groups showed non-significant variation, the overall trend demonstrated that microbial inoculation, especially with SOB12, contributed substantially to increased pigment biosynthesis under reduced sulfur application.

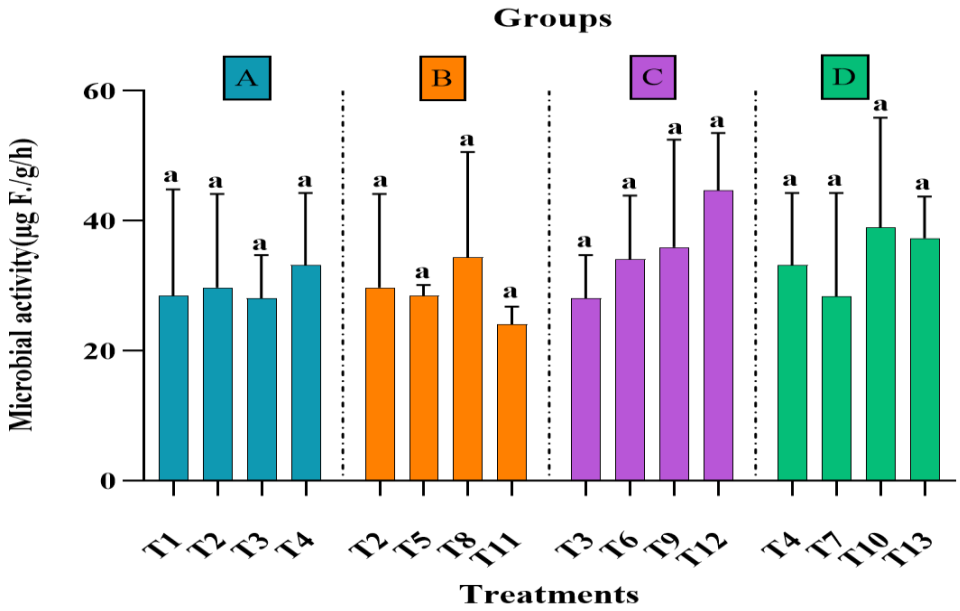

Fig. 2. Microbial activity (µg F./g/h) by Fluorescein Diacetate (FDA) hydrolysis in soil at harvest under different SOB treatments and sulfur levels.

Grouping for one-way ANOVA: Group **A** (no inoculation across sulfur levels), Group **B** (50% sulfur across inoculation types), Group **C** (100% sulfur across inoculation types), Group **D** (150% sulfur across inoculation types).
$\mathbf{T_1}$:Control (NPK only), $\mathbf{T_2}$: NPK+50%S, $\mathbf{T_3}$:NPK+100%S, $\mathbf{T_4}$:NPK+150%S,$\mathbf{T_5}$:NPK+50%S +(SOB12), $\mathbf{T_6}$: NPK+100%S +(SOB12), $\mathbf{T_7}$:NPK+150% S+(SOB12),$\mathbf{T_8}$:NPK+50% S+(SOB15), $\mathbf{T_9}$: NPK+100% S+(SOB15),$\mathbf{T_{10}}$:NPK+150% S+(SOB15), $\mathbf{T_{11}}$:NPK+50% S+(12&15 SOB), $\mathbf{T_{12}}$: NPK+100%S+(12&15 SOB),$\mathbf{T_{13}}$:NPK+150%S+(12&15 SOB).

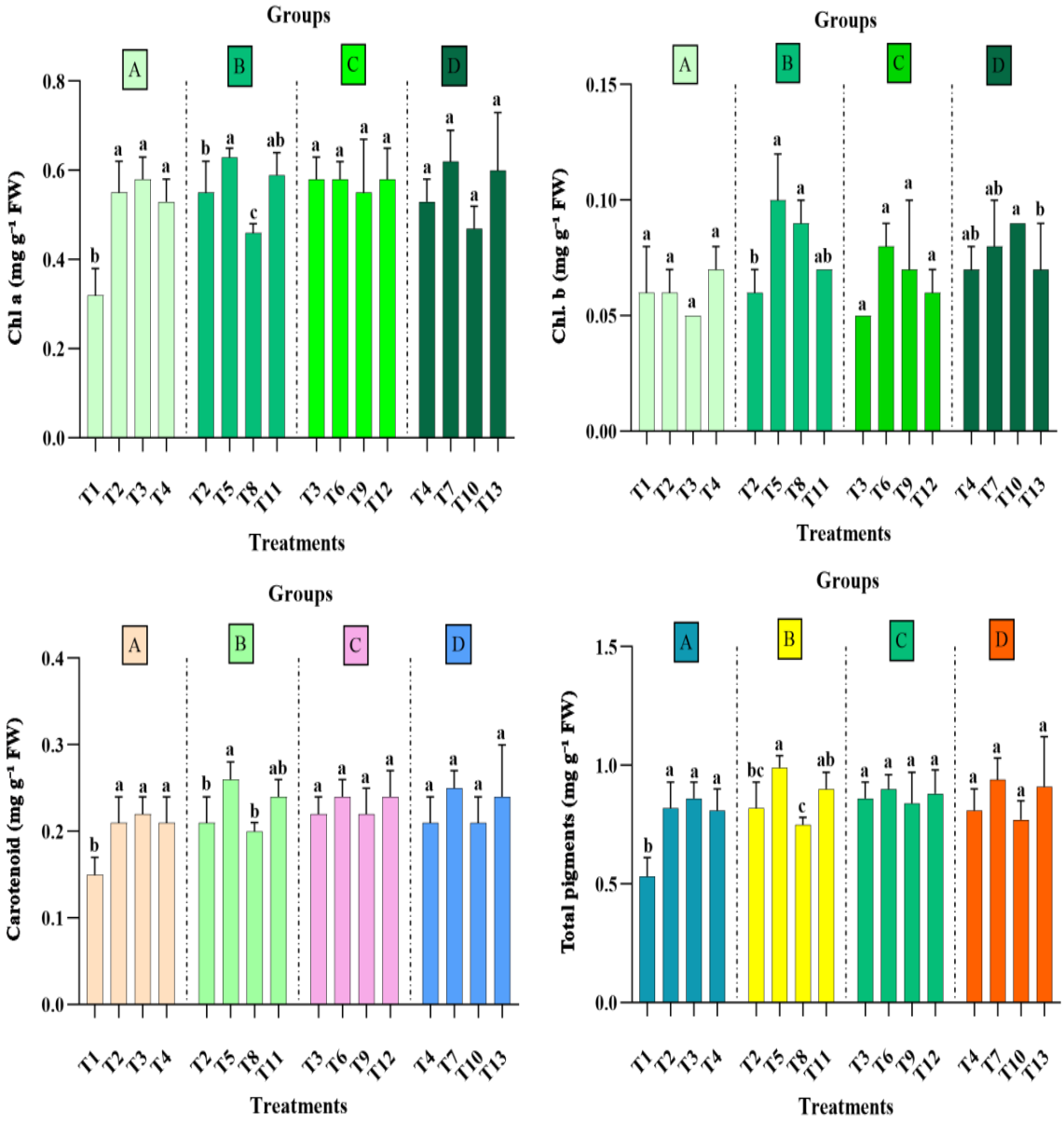

Fig. 3. Chlorophyll a, b, carotenoid and total pigments content in onion leaves at 100 days under different SOB treatments and sulfur levels.

Grouping for one-way ANOVA: Grouping for one-way ANOVA: Group **A** (no inoculation across sulfur levels), Group **B** (50% sulfur across inoculation types), Group **C** (100% sulfur across inoculation types), Group **D** (150% sulfur across inoculation types).

T_1:Control(NPKonly),**T_2**:NPK+50%S,**T_3**:NPK+100%S,**T_4**:NPK+150%S,**T_5**:NPK+50%S+(SOB12),**T_6**:NPK+100%S+(SOB12),**T_7**:NPK+150%S+(SOB12),**T_8**:NPK+50%S+(SOB15),**T_9**:NPK+100%S+(SOB15),**T_{10}**:NPK+150%S+(SOB15),**T_{11}**:NPK+50%S+(12&15SOB),**T_{12}**:NPK+100%S+(12&15SOB),**T_{13}**:NPK+150%S+(12&15 SOB).

3.6. Effect of sulfur-oxidizing bacteria and sulfur levels on vegetative growth and bulb morphology of onion plants

As shown in Table 4, clear differences were observed in onion plant growth traits in response to sulfur application and bacterial inoculation. In the control group (A), applying sulfur without microbial inoculants led to a gradual and significant increase in plant length, with the highest value (68.70 cm) recorded under 150% sulfur. Bulb dimensions were also enhanced in this group, particularly bulb length, which increased from 2.58 cm in the control to 4.23 cm under 150% sulfur, while neck characteristics showed limited variation. These findings indicate that sulfur alone, even in the absence of bioinoculants, improves vegetative and bulb growth to a notable extent (Table 4).

Table 4. Effect of sulfur-oxidizing bacterial inoculation and sulfur concentrations on plant height, number of leaves, bulb dimensions (length and diameter), and neck dimensions (length and diameter) of onion plants at harvest.

Groups	Treatments	Plant length (cm)	No. Leaves	Bulb (cm)		Neck (cm)	
				length	diameter	length	diameter
A	T_1	53.68c	8.60b	2.58b	2.22b	6.60b	1.26a
	T_2	63.45b	9.80ab	4.28a	2.82a	9.48a	1.67a
	T_3	66.00ab	10.60a	4.40a	2.78a	9.08a	1.45a
	T_4	68.70a	9.20ab	4.23a	3.22a	8.73a	1.73a
	F test	***	*	***	***	***	NS
B	T_2	63.45c	9.80a	4.28a	2.82b	9.48a	1.67b
	T_5	74.73b	10.80a	4.40a	4.35a	9.43a	2.83a
	T_8	87.00a	10.60a	4.25a	3.71a	8.13a	2.41a
	T_{11}	83.45a	10.00a	4.33a	3.94a	8.75a	2.36a
	F test	***	NS	NS	***	NS	***
C	T_3	66.00b	10.60a	4.40a	2.78b	9.08a	1.45b
	T_6	77.05ab	11.00a	4.48a	3.43a	9.03a	1.83a
	T_9	81.30a	9.40a	4.33a	3.60a	8.48a	1.75ab
	T_{12}	80.98a	9.60a	4.40a	3.32ab	9.28a	1.88a
	F test	*	*	NS	*	NS	*
D	T_4	68.70b	9.20b	4.23b	3.22a	8.73b	1.73a
	T_7	82.53a	10.80a	4.58ab	3.98a	10.38a	1.89a
	T_{10}	82.60a	11.60a	5.00a	4.00a	8.75b	1.90a
	T_{13}	81.03a	10.80a	4.40b	3.98a	8.60b	1.78a
	F test	***	**	**	NS	*	NS

Values are presented as mean values (n = 5 replicates). Different letters within the same group indicate significant differences at $p \leq 0.05$ according to Tukey's test. F test: NS = not significant, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. Grouping for one-way ANOVA: Group A (no inoculation across sulfur levels), Group B (50% sulfur across inoculation types), Group C (100% sulfur across inoculation types), Group D (150% sulfur across inoculation types).

T_1:Control (NPK only), T_2: NPK+50%S, T_3:NPK+100%S, T_4:NPK+150%S,T_5:NPK+50%S +(SOB12), T_6: NPK+100%S +(SOB12), T_7:NPK+150% S+(SOB12),T8:NPK+50% S+(SOB15), T_9: NPK+100% S+(SOB15),T_{10}:NPK+150% S+(SOB15), T_{11}:NPK+50% S+(12&15 SOB), T_{12}: NPK+100%S+(12&15 SOB),T_{13}:NPK+150%S+(12&15 SOB).

With the addition of bacterial inoculants alongside 50% sulfur (Group B), the response was more pronounced in shoot elongation, especially with *Priestia aryabhattai* (SOB15), which produced the tallest plants (87.00 cm). Co-inoculation also yielded comparably high values (83.45 cm), suggesting a synergistic effect on plant height. Although bulb traits in this group did not vary significantly, neck diameter showed a marked increase under inoculated treatments, particularly in the dual-inoculated plants (2.36–2.83 cm), reinforcing the role of microbes in enhancing tissue expansion under limited sulfur input (Table 4).

In Group C, combining 100% sulfur with bacterial inoculants led to further improvements. Plant height reached 81.30 cm with SOB15 and remained consistently higher in other inoculated treatments compared to the uninoculated one. Neck diameter also increased, peaking at 1.88 cm in the co-inoculated treatment. Despite minimal variation in bulb length and diameter, the enhanced vertical growth and neck development suggest that microbial biofertilization is effective even at moderate sulfur levels (Table 4). Similarly, in Group D, where 150% sulfur was coupled with microbial inoculation, both plant height and bulb dimensions improved. SOB15 again produced the tallest plants (82.60 cm), and the largest bulb length (5.00 cm), indicating that this strain consistently supports shoot and bulb growth under elevated sulfur availability. While not all differences were statistically significant, the observed patterns across treatments support the potential benefit of combining high sulfur rates with targeted microbial inoculants (Table 4).

3.7. Effect of sulfur-oxidizing bacteria and sulfur levels on N, P, and K uptake in onion bulbs

As shown in Table 5, microbial inoculation and sulfur application markedly affected the uptake of nitrogen, phosphorus, and potassium in onion bulbs. In the control group (A), a clear enhancement in nutrient uptake was observed with increasing sulfur levels. Nitrogen content rose from 0.059 g plant^{-1} in the untreated control to 0.141 g plant^{-1} at 100% sulfur, while phosphorus increased fourfold from 0.002 to 0.008 g plant^{-1}. Potassium uptake showed a similar pattern, reaching its peak (0.105 g plant^{-1}) at 100% sulfur before declining slightly at 150%. These results confirm the stimulatory effect of sulfur on macronutrient accumulation even in the absence

of microbial inoculants (Table 5). Group B, in which 50% sulfur was combined with microbial inoculation, demonstrated improved nutrient uptake compared to sulfur alone. The highest nitrogen value (0.191 g plant^{-1}) was recorded with *Priestia aryabhattai* (SOB15), and the mixed inoculum also showed elevated N and K levels, though without significant differences.

Phosphorus uptake was significantly higher with *Bacillus spizizenii* (SOB12), reaching 0.017 g plant^{-1}, which may reflect increased solubilization under microbial influence. Despite variability among treatments, these results highlight the role of bacteria in enhancing nutrient availability when applied under sub-optimal sulfur conditions (Table 5). Further improvements were evident in Group C, where 100% sulfur was paired with inoculants. Nitrogen uptake peaked at 0.204 g plant^{-1} with SOB15, and remained high in other inoculated treatments. Potassium values ranged from 0.105 to 0.133 g plant^{-1}, indicating that microbial inoculation enhanced potassium uptake efficiency. Although phosphorus uptake was modest, the combined effects of 100% sulfur and bioinoculants led to consistently better nutrient profiles compared to uninoculated controls within this group (Table 5).

In Group D, which combined 150% sulfur with inoculation, nitrogen and potassium uptake reached their highest values across all treatments. The SOB15 treatment yielded the maximum nitrogen uptake (0.242 g plant^{-1}) and one of the highest potassium values (0.151 g plant^{-1}), reinforcing the consistent performance of this strain. Mixed inoculation, however, showed a reduction in all three elements compared to single-strain treatments, suggesting a possible antagonistic interaction or nutrient competition under high sulfur availability (Table 5).

Overall, nitrogen and potassium uptake showed stronger and more consistent responses to bacterial inoculation than phosphorus. Among all treatments, the combination of *Priestia aryabhattai* with 150% sulfur exhibited the most pronounced increase in nitrogen accumulation, while potassium followed a similar trend, albeit with less statistical distinction. These results underscore the role of sulfur-oxidizing bacteria, especially SOB15, in maximizing nutrient uptake in onion plants under varying sulfur conditions.

Table 5. Effect of sulfur-oxidizing bacterial inoculation and sulfur concentrations on nitrogen (N), phosphorus (P), and potassium (K) uptake (g plant^{-1}) in onion bulbs at harvest.

Groups	Treatments	Macronutrient Analysis in Onion Bulbs		
		N Uptake (g plant^{-1})	P Uptake (g plant^{-1})	K Uptake (g plant^{-1})
A	T_1	0.059b	0.002b	0.067b
	T_2	0.125a	0.004ab	0.098a
	T_3	0.141a	0.008a	0.105a
	T_4	0.117a	0.007a	0.085ab
	F test	**	*	*
B	T_2	0.125a	0.004c	0.098a
	T_5	0.149a	0.017a	0.111a
	T_8	0.191a	0.015ab	0.124a
	T_{11}	0.163a	0.009bc	0.119a
	F test	NS	**	NS
C	T_3	0.141b	0.008b	0.105b
	T_6	0.189a	0.018a	0.126ab
	T_9	0.204a	0.013ab	0.133a
	T_{12}	0.181ab	0.012ab	0.125ab
	F test	*	*	*
D	T_4	0.117c	0.007a	0.085b
	T_7	0.208ab	0.012a	0.143a
	T_{10}	0.242a	0.015a	0.151a
	T_{13}	0.158bc	0.009a	0.092b
	F test	***	NS	**

Values are presented as mean values (n = 5 replicates). Different letters within the same group indicate significant differences at $p \leq 0.05$ according to Tukey's test. F test : NS = not significant, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. Grouping for one-way ANOVA: Group A (no inoculation across sulfur levels), Group B (50% sulfur across inoculation types), Group C (100% sulfur across inoculation types), Group D (150% sulfur across inoculation types).
T_1:Control (NPK only), T2:NPK+50%S, T_3:NPK+100%S, T_4:NPK+150%S,T_5:NPK+50%S +(SOB12),T_6:NPK+100%S+(SOB12),T_7:NPK+150%S+(SOB12),T_8:NPK+50%S+(SOB15),T_9:NPK+100%S+(SOB15),T_{10}: NPK+150%S+(SOB15),T_{11}:NPK+50%S+(12&15SOB),T_{12}:NPK+100%S+(12&15SOB),T_{13}:NPK+150%S+(12&15 SOB).

3.8. Sulfur content in o bulbs as affected by bacterial treatments and sulfur doses

Variations in total sulfur content in onion bulbs under different treatments are illustrated in Fig 4. In the control group (A), sulfur concentration increased steadily with sulfur application, ranging from 0.042% in the uninoculated control to 0.125% with 100% sulfur. The increase from 50% to 150% sulfur was marginal, indicating a plateau effect beyond the optimal level in the absence of microbial support. A closer look at Group B reveals that microbial inoculation influenced sulfur accumulation even under fixed sulfur input (50%). While the uninoculated treatment showed 0.104%, co-inoculated plants exhibited a higher sulfur content (0.122%), although the differences among treatments were moderate. These results suggest that sulfur-oxidizing bacteria may assist in improving sulfur assimilation efficiency under low sulfur conditions.

Further enhancement was noted in Group C, where inoculation was combined with 100% sulfur. The dual inoculation treatment resulted in the highest sulfur concentration of all treatments (0.167%), exceeding the single-strain inoculants and the uninoculated control. This outcome reflects a potential synergistic interaction between microbial activity and adequate sulfur availability. In contrast, the treatments in Group D, receiving 150% sulfur, displayed more consistent sulfur concentrations across inoculated plants, with values ranging between 0.126% and 0.136%. While still elevated compared to their uninoculated counterpart (0.123%), the differences were not statistically significant, indicating that the maximum sulfur concentration may have already been approached under this higher input. Together, the findings from Fig. 4 highlight that sulfur accumulation in onion bulbs responds positively to both increased sulfur supply and microbial inoculation, with the most pronounced effect observed in co-inoculated plants receiving 100% sulfur. These data further support the role of sulfur-oxidizing bacteria in enhancing the bioavailability and uptake of sulfur, particularly when the applied rate matches plant demand.

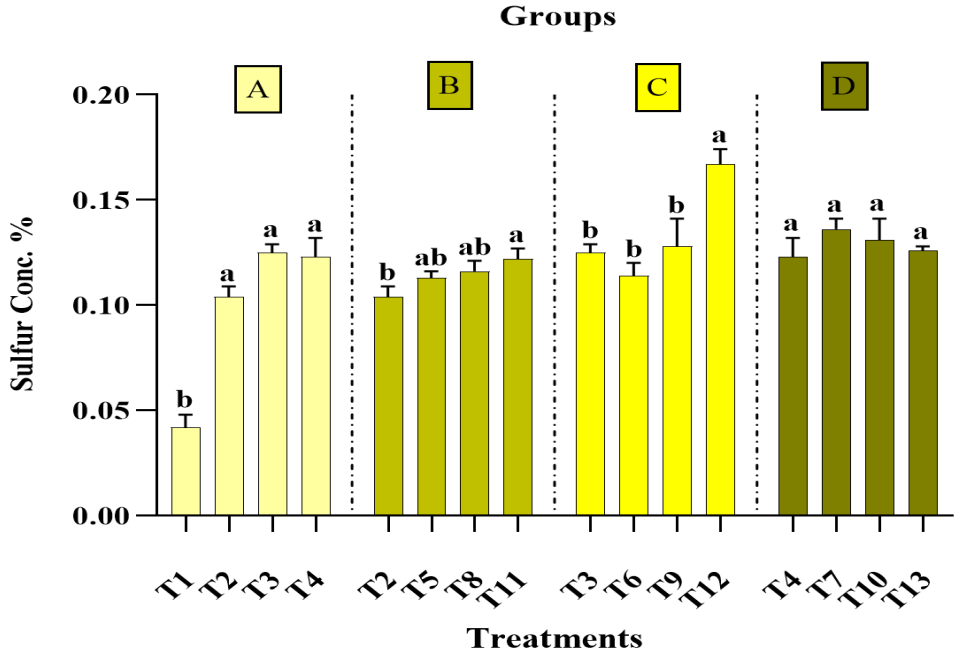

Fig. 4. Sulfur concentrations in onion bulbs at harvest under different SOB treatments and sulfur levels.

Grouping for one-way ANOVA: Group A (no inoculation across sulfur levels), Group B (50% sulfur across inoculation types), Group C (100% sulfur across inoculation types), Group D (150% sulfur across inoculation types).
T_1:Control (NPK only), T_2: NPK+50%S, T_3:NPK+100%S, T_4:NPK+150%S,T_5:NPK+50%S +(SOB12), T_6: NPK+100%S +(SOB12), T_7:NPK+150% S+(SOB12),T_8:NPK+50% S+(SOB15), $\mathbf{T_9}$: NPK+100% S+(SOB15),$\mathbf{T_{10}}$:NPK+150% S+(SOB15), $\mathbf{T_{11}}$:NPK+50% S+(12&15 SOB), $\mathbf{T_{12}}$: NPK+100%S+(12&15 SOB),$\mathbf{T_{13}}$:NPK+150%S+(12&15 SOB).

3.9. Effect of sulfur-oxidizing bacteria and sulfur on biochemical constituents in onion bulbs

The concentration of pyruvic acid in onion bulbs varied notably across treatments and groups, reflecting differences in metabolic activity and pungency potential (Table 6). In the control group (A), sulfur addition increased pyruvic acid levels from 15.80 to 25.61 mmol/g FW, with a significant jump starting at 100% sulfur.

Table 6. Effect of sulfur-oxidizing bacterial inoculation and sulfur concentrations on pyruvic acid (mmol g^{-1} FW), total phenolic content (µg g^{-1} FW), total carbohydrate concentration (mg g^{-1} DW), and free amino acid content (mg g^{-1} DW) in onion bulbs at harvest.

Groups	Treatments	Biochemical Constituents			
		Pyruvic acid conc. (mmol g-1 FW)	Total phenolic (µg g^{-1} FW)	Carbohydrate conc. (mg g^{-1} DW)	Free Amino acid conc. (mg g^{-1} DW)
A	T_1	15.80b	0.35b	0.93c	111.52c
	T_2	18.56b	0.62a	2.13b	195.83b
	T_3	24.85a	0.67a	3.47a	218.00b
	T_4	25.61a	0.67a	3.14ab	353.10a
	F test	***	**	***	***
B	T_2	18.56c	0.62b	2.13b	195.83b
	T_5	26.71a	0.93a	4.95a	316.05b
	T_8	23.31b	0.78ab	4.17a	486.17a
	T_{11}	23.23b	0.86a	4.08a	281.03b
	F test	***	**	***	***
C	T_3	24.85a	0.67a	3.47a	218.00b
	T_6	20.98a	0.80a	6.69a	247.34b
	T_9	20.67a	0.75a	3.41a	382.62a
	T_{12}	22.62a	0.88a	4.10a	248.23b
	F test	NS	NS	*	***
D	T_4	25.61a	0.67a	3.14b	353.10a
	T_7	24.11a	0.82a	5.08ab	170.83b
	T_{10}	22.59a	0.70a	7.73a	340.51a
	T_{13}	20.34a	0.83a	7.92a	296.37ab
	F test	NS	NS	**	**

Values are presented as mean values (n = 5 replicates). Different letters within the same group indicate significant differences at $p \leq 0.05$ according to Tukey's test. F test : NS = not significant, * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$. Grouping for one-way ANOVA: Group A (no inoculation across sulfur levels), Group B (50% sulfur across inoculation types), Group C (100% sulfur across inoculation types), Group D (150% sulfur across inoculation types).
T_1:Control (NPK only), T_2: NPK+50%S, T_3:NPK+100%S, T_4:NPK+150%S,T_5:NPK+50%S +(SOB12), T_6: NPK+100%S +(SOB12), T_7:NPK+150% S+(SOB12),T8:NPK+50% S+(SOB15), T_9: NPK+100% S+(SOB15),T_{10}:NPK+150% S+(SOB15), T_{11}:NPK+50% S+(12&15 SOB), T_{12}: NPK+100%S+(12&15 SOB),T_{13}:NPK+150%S+(12&15 SOB).

Inoculated plants in Group C displayed more stable pyruvic acid values across treatments, without significant differences, although all values remained above 20 mmol g^{-1}. In Group D, concentrations slightly declined with inoculation, but values remained within the higher range, particularly in the SOB12 treatment (24.11 mmol g^{-1} FW). These patterns indicate that both sulfur and microbial inoculation enhance pyruvic acid accumulation, with the most pronounced effect seen under SOB12 at 50% sulfur. As shown in Table 6, total phenolic content increased consistently in response to both sulfur application and microbial inoculation. In Group A, values nearly doubled from 0.35 to 0.67 µg g^{-1} FW with the application of sulfur alone. In Group B, phenolic content peaked at 0.93 µg g^{-1} FW with SOB12, followed closely by the dual inoculum treatment (0.86 µg g^{-1}). Although differences were not statistically significant in Group C, there was a gradual rise across treatments, with the highest value (0.88 µg g^{-1}) observed under co-inoculation. Similarly, Group D maintained a relatively narrow range of values, with inoculated treatments showing higher phenolic concentrations than the uninoculated control. These findings suggest a microbial role in promoting antioxidant accumulation, particularly under low-to-moderate sulfur levels.

Turning to carbohydrate content, sulfur application and microbial treatments significantly impacted carbohydrate accumulation in the bulbs (Table 6). In the control group, carbohydrates increased from 0.93 to 3.47 mg g^{-1} DW with rising sulfur levels. Group B exhibited even higher values, with SOB12 treatment yielding nearly 5 mg g^{-1} and SOB15 reaching 4.17 mg g^{-1}. In Group C, the highest carbohydrate content (6.69 mg g^{-1}) was observed under SOB12 at 100% sulfur, surpassing the uninoculated treatment. Group D showed the most substantial accumulation, particularly in SOB15 and co-inoculated plants, which reached values exceeding 7.7 mg g^{-1}.

These results highlight the synergistic effect of microbial inoculation and high sulfur input in enhancing carbohydrate biosynthesis.

The data in Table 6 also reveal strong differences in free amino acid concentrations among treatments. In Group A, sulfur supplementation led to a sharp rise in amino acid content, particularly at 150% sulfur, where the value reached 353.10 mg g^{-1} DW more than three times the control level. In Group B, SOB15 treatment resulted in the highest amino acid level (486.17 mg g^{-1}), indicating a strong stimulatory effect. In Group C, plants treated with SOB15 also showed the greatest accumulation (382.62 mg g^{-1}), whereas in Group D, high values were maintained across inoculated treatments, peaking under the uninoculated condition. The consistent trend across groups supports the role of sulfur and beneficial bacteria in stimulating nitrogen metabolism and amino acid biosynthesis.

3.10. Total protein percentage in onion bulbs

The percentage of total protein in onion bulbs responded variably to the treatments applied (Fig 5). In Group A, sulfur supplementation resulted in a nearly twofold increase in protein content, rising from 2.54%

in the NPK-only control to 5.69% with 50% sulfur. The values remained high with 100% sulfur (5.43%) but slightly declined at 150% (4.49%), suggesting an optimal protein response at moderate sulfur levels in the absence of microbial inoculants. Group B showed further enhancement with bacterial application. The highest protein level (5.89%) was recorded with *Priestia aryabhattai* (SOB15), followed by the co-inoculated treatment (5.40%). Interestingly, SOB12 produced a moderate increase (4.67%), while the uninoculated treatment (50% sulfur alone) recorded the lowest value in this group (4.29%). These differences indicate a clear role for SOB15 in promoting protein biosynthesis, even at lower sulfur levels. In Group C, protein content remained relatively consistent across treatments, ranging from 5.43% to 5.78%. No statistically significant differences were detected, yet the highest value was observed in the dual inoculation treatment. In Group D, co-inoculation again resulted in the highest protein content (6.48%), closely followed by SOB15 (6.24%). These values surpass all other treatments, highlighting a potential synergistic effect between elevated sulfur and microbial combinations. Overall, while differences were not always statistically significant, the trend suggests that combining 150% sulfur with SOB15 or mixed inoculation yields the greatest enhancement in protein content. This reinforces the beneficial impact of sulfur-oxidizing bacteria, particularly under high sulfur availability, in boosting the nutritional quality of onion bulbs.

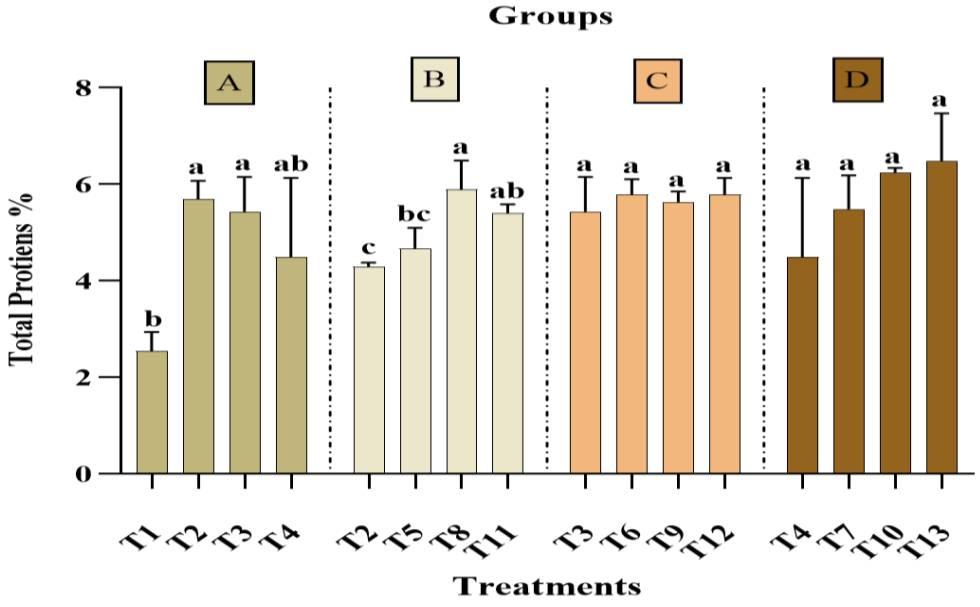

Fig. 5. Total protein content (%) in onion bulbs at harvest under different SOB treatments and sulfur levels.

Grouping for one-way ANOVA: Group **A** (no inoculation across sulfur levels), Group **B** (50% sulfur across inoculation types), Group **C** (100% sulfur across inoculation types), Group **D** (150% sulfur across inoculation types).**T_1**:Control(NPK only),**T_2**: NPK+50%S, **T_3**:NPK+100%S, **T_4**:NPK+150%S,**T_5**:NPK+50%S +(SOB12), **T_6**: NPK+100%S +(SOB12), **T_7**:NPK+150% S+(SOB12),**T8**:NPK+50% S+(SOB15), **T_9**: NPK+100% S+(SOB15),**T_{10}**:NPK+150% S+(SOB15), **T_{11}**:NPK+50% S+(12&15 SOB), **T_{12}**: NPK+100%S+(12&15 SOB),**T_{13}**:NPK+150%S+(12&15 SOB).

4. Discussion

The present study provides comprehensive insights into the effects of sulfur-oxidizing bacteria (SOB), namely *Bacillus spizizenii* (SOB12) and *Priestia aryabhattai* (SOB15), in combination with graded sulfur levels, on the growth performance, nutrient uptake, and biochemical quality of onion (*Allium cepa* L.) plants. Results from the biomass assessment clearly indicate that both sulfur application and SOB inoculation enhanced fresh and dry weights of onion shoots and bulbs. Notably, the co-inoculation of SOB12 and SOB15 with 50% sulfur significantly increased bulb dry weight (4.23 g plant^{-1}), suggesting a synergistic effect in enhancing sulfur utilization under sub-optimal sulfur conditions. Similar findings were reported by Awad et al. (2011), where SOBs increased yield through improved nutrient mobilization and sulfur assimilation. Photosynthetic pigment content, including chlorophyll a, b, and carotenoids, was markedly improved under SOB inoculation. Treatments with SOB15 under 100% sulfur produced the highest pigment levels, particularly chlorophyll a (0.63 mg g^{-1} FW) and total pigments (0.93 mg g^{-1} FW). This enhancement is likely due to increased sulfur and nitrogen availability, as sulfur is essential in chlorophyll biosynthesis and protein metabolism (Zenda et al., 2021; Shah et al., 2022). The ability of SOBs to release sulfate and improve rhizospheric conditions may have contributed to elevated pigment production, even at lower sulfur levels.

Vegetative growth traits, including plant height and bulb dimensions, showed consistent improvements across inoculated treatments. In particular, plants inoculated with SOB15 at 50% sulfur reached the highest plant height (87.00 cm), while co-inoculated plants achieved notable increases in bulb and neck diameter. These effects support the role of beneficial microbes in promoting cell expansion and vegetative vigor by enhancing sulfur availability and overall nutrient uptake (Rana et al., 2020). Moreover, SOBs are known to enhance soil microbial diversity and enzymatic activity, which may further stimulate plant development (Ranadev et al., 2023). The superior performance of SOB15 over SOB12 in several growth and quality parameters may be attributed to strain-specific functional traits. SOB15 (*Priestia aryabhattai*) is reported to possess higher sulfur oxidation efficiency, greater tolerance to environmental stress, and the ability to produce plant growth-promoting metabolites such as indole-3-acetic acid (IAA), siderophores, and ACC deaminase (Kumar et al., 2024; Vishwakarma et al., 2024). These traits enhance nutrient solubilization and alleviate stress conditions, thereby supporting improved plant biomass and biochemical composition. By contrast, SOB12 (*Bacillus spizizenii*), although effective in sulfur oxidation, may have lower expression of such growth-promoting mechanisms, which could explain the differential effects observed across traits. Such strain-dependent variations highlight the importance of selecting and combining complementary microbial inoculants to maximize crop performance (Kong and Liu, 2022; Mao et al., 2023).

Macronutrient analysis revealed substantial increases in N, P, and K uptake in onion bulbs due to SOB application. SOB15 at 150% sulfur achieved the highest nitrogen uptake (0.242 g plant^{-1}), while phosphorus was significantly elevated under SOB12 inoculation at 50% sulfur (0.017 g plant^{-1}). These results are in line with previous reports indicating that SOBs not only oxidize sulfur but also solubilize phosphorus and mobilize potassium, contributing to improved nutrient acquisition (Vishnu et al., 2024; Vidyalakshmi et al., 2009). Sulfur content in bulbs was also enhanced by SOB treatments. The highest sulfur accumulation (0.167%) was observed in dual-inoculated plants under 100% sulfur, demonstrating the ability of SOBs to increase sulfur bioavailability and assimilation. Even under reduced sulfur input (50%), co-inoculated plants exhibited elevated sulfur levels compared to uninoculated controls. These findings underscore the efficiency of SOBs in converting elemental sulfur to sulfate, the plant-available form, especially under moderate application rates (El-Bakry et al., 2024; Yebalework et al., 2024).

Biochemical analyses further highlighted the benefits of microbial inoculation. Pyruvic acid concentrations, associated with onion pungency and sulfur metabolism, peaked in SOB12-inoculated plants at 50% sulfur (26.71 mmol g^{-1} FW), suggesting efficient sulfur assimilation at reduced inputs. Additionally, total phenolics, known for their antioxidant properties, were highest in SOB12 and dual inoculation treatments, particularly under 50–100% sulfur. This is consistent with studies showing that SOBs enhance the accumulation of secondary metabolites by improving sulfur and nitrogen supply (Sagar et al., 2022; Dalamu et al., 2010).

Carbohydrate content increased significantly in response to combined sulfur and SOB treatment. Maximum levels (>7.7 mg g^{-1} DW) were recorded in SOB15 and co-inoculated plants under 150% sulfur, reflecting enhanced photosynthetic production and storage. Likewise, free amino acids and total soluble proteins were elevated across all SOB treatments, with SOB15 and co-inoculation showing the highest values under high sulfur availability. These improvements point to the stimulatory effects of SOBs on nitrogen metabolism and protein biosynthesis, as also noted by Muscolo et al. (2025).

Interestingly, dual inoculation did not always outperform single-strain treatments under high sulfur availability (e.g., 150% sulfur). This underperformance may be explained by competitive interactions between SOB12 and SOB15 in the rhizosphere, where both strains simultaneously compete for sulfur substrates and root exudates, potentially reducing their individual efficiency (Wang and Kuzyakov, 2024). Moreover, under conditions of abundant sulfur, the advantage of microbial oxidation becomes less critical, and the additional energy cost of

maintaining two active microbial populations could limit their collective benefits (Singh et al., 2022). Similar findings have been reported in studies where multi-strain inoculants did not consistently exceed single inoculations, highlighting the importance of compatibility and ecological balance among strains (Thomloudi et al., 2019; Kumar et al., 2024). These results suggest that while dual inoculation is beneficial under limited nutrient supply, its effectiveness may decline under nutrient-rich conditions due to microbial competition and resource redundancy.

Importantly, despite the improved performance at 150% sulfur, many parameters achieved comparable or superior results at 50% sulfur when combined with SOB inoculation. This suggests that microbial enhancement allows for a reduction in chemical fertilizer inputs without compromising plant productivity or quality, aligning with sustainable agriculture goals. While the present pot experiment clearly demonstrated the beneficial role of sulfur-oxidizing bacteria in enhancing onion growth and quality traits, some limitations should be acknowledged. Environmental variables under field conditions, such as soil heterogeneity, temperature fluctuations, and moisture levels, may influence the efficacy of SOB and should be taken into account when extrapolating these findings. Therefore, further field trials across different agro-ecological zones are warranted to validate the consistency of the observed responses. In addition, long-term studies are recommended to evaluate how repeated SOB application may impact soil health, nutrient cycling, and overall crop productivity over multiple growing seasons. These future directions would help to better establish SOB as a sustainable biofertilizer strategy in commercial onion cultivation.

5. Conclusions

The present study demonstrated that inoculation with sulfur-oxidizing bacteria *Bacillus spizizenii* (SOB12) and *Priestia aryabhattai* (SOB15) significantly enhanced the growth, nutrient uptake, and biochemical properties of onion plants. The most effective treatments were those combining either or both bacterial strains with 50% of the recommended sulfur dose, highlighting a synergistic interaction that supports reduced chemical input. These findings underline the potential of SOBs as sustainable biofertilizers capable of improving onion productivity and quality under limited sulfur conditions, and promote their application in environmentally friendly agricultural practices. The findings highlight the practical potential of SOB inoculants as biofertilizers that can reduce chemical sulfur fertilizer requirements by up to 50% without compromising yield or quality. This has direct implications for onion cultivation, as it can lower input costs for farmers, improve soil health, and minimize the environmental footprint associated with excessive fertilizer use. Moreover, the strain-specific benefits observed, particularly with SOB15, emphasize the importance of selecting efficient microbial inoculants tailored to crop and soil conditions. These outcomes strongly support the integration of SOB into sustainable agricultural practices, where microbial biofertilization contributes to enhanced crop productivity, resource-use efficiency, and long-term soil fertility.

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References

Adam, G., & Duncan, H. (2001). Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. *Soil biology and biochemistry*, *33*(7-8), 943-951.

Awad, N. M., Abd El-Kader, A. A., Attia, M. K. A. A., & Alva, A. K. (2011). Effects of nitrogen fertilization and soil inoculation of sulfur- oxidizing or nitrogen- fixing bacteria on onion plant growth and yield. *International Journal of Agronomy*, *2011*(1), 316856.

Dalamu, Kaur, C., Singh, M., Walia, S., Joshi, S., & Munshi, A. D. (2010). Variations in phenolics and antioxidants in Indian onions (*Allium cepa* L.) Genotype selection for breeding. *Nutrition & Food Science*, *40*(1), 6-19.

DuBois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A., & Smith, F. (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, *28*(3), 350-356.

El-Bakry, F. A., El-Sonbaty, A. E., El-Sherpiny, M. A., & Hashish, N. R. (2024). Synergistic Effects of Elemental Sulfur Soil Addition and Beneficial Elements Spraying on Onions. *Journal of Soil Sciences and Agricultural Engineering*, *15*(6), 135-141.

Elenany, A. M., Atia, M. M. M., Abbas, E. E., Moustafa, M., Alshaharni, M. O., Negm, S., & Elnahal, A. S. M. A. (2024). Nanoparticles and chemical inducers: A sustainable shield against onion white rot. *Biology*, *13*(4), 219.

Hymowitz, T., Collins, F. I., Panczner, J., & Walker, W. M. (1972). Relationship between the content of oil, protein, and sugar in soybean seed 1. *Agronomy Journal*, *64*(5), 613-616.

Jackson, M. L. (1973). Soil chemical analysis, pentice hall of India Pvt. *Ltd., New Delhi, India*, *498*, 151-154.

Kong, Z., & Liu, H. (2022). Modification of rhizosphere microbial communities: a possible mechanism of plant growth promoting rhizobacteria enhancing plant growth and fitness. *Frontiers in Plant Science*, *13*, 920813.

Kumar, C., Esposito, A., Bertani, I., Musonerimana, S., Midekssa, M. J., Tesfaye, K., & Venturi, V. (2024). Sorghum rhizosphere bacteriome studies to pinpoint, isolate and assess plant beneficial bacteria. *Isolate and Assess Plant Beneficial Bacteria*. PREPRINT (Version 1) available at Research Square

Lee, Y. P., & Takahashi, T. (1966). An improved colorimetric determination of amino acids with the use of ninhydrin. *Analytical biochemistry*, *14*(1), 71-77.

Lichtenthaler, H. K. (1987). Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. In *Methods in enzymology* (Vol. 148, pp. 350-382). Academic Press.

Luo, D., Tian, B., Li, J., Zhang, W., Bi, S., Fu, B., & Jing, Y. (2024). Mechanisms underlying the formation of main volatile odor sulfur compounds in foods during thermal processing. *Comprehensive Reviews in Food Science and Food Safety*, *23*(4), e13389.

Ma, L., Zhao, R., Li, J., Yang, Q., & Liu, Y. (2024). Release characteristics and risk assessment of volatile sulfur compounds in a municipal wastewater treatment plant with odor collection device. *Journal of Environmental Management*, *354*, 120321.

Majid, I., Majid, D., Makroo, H. A., & Dar, B. N. (2024). Enhancing the bioavailability and gut health benefits of quercetin from sprouted onions: A comprehensive review in the context of food-derived bioactives. *Food Chemistry Advances*, *4*, 100725.

Mao, Y., Chang, D., Cui, X., Wu, Y., & Cai, B. (2023). Changes in sulfur in soybean rhizosphere soil and the response of microbial flora in a continuous cropping system mediated by Funneliformis mosseae. *Frontiers in Microbiology*, *14*, 1235736.

Mu, X., Yin, S., Su, F., Jiang, R., Liang, S., Liu, Y., & Li, N. (2024). Understanding the Onion Lachrymatory Factor: Structure, Formation, and Flavor Implications. *Food Reviews International*, *40*(10), 3938-3963.

Muscolo, A., Maffia, A., Marra, F., Battaglia, S., Oliva, M., Mallamaci, C., & Russo, M. (2025). Unlocking the Health Secrets of Onions: Investigating the Phytochemical Power and Beneficial Properties of Different Varieties and Their Parts. *Molecules*, *30*(8), 1758.

Ochar, K., & Kim, S. H. (2023). Conservation and global distribution of onion (*Allium cepa* L.) germplasm for agricultural sustainability. *Plants*, *12*(18), 3294.

Pratt, P. F., & Chapman, H. D. (1961). Gains and losses of mineral elements in an irrigated soil during a 20-year lysimeter investigation. *Hilgardia*, *30*(16), 445-67

Rana, K., Rana, N., & Singh, B. (2020). Applications of sulfur oxidizing bacteria. In *Physiological and biotechnological aspects of extremophiles* (pp. 131-136). Academic Press.

Ranadev, P., Revanna, A., Bagyaraj, D. J., & Shinde, A. H. (2023). Sulfur oxidizing bacteria in agro ecosystem and its role in plant productivity—a review. *Journal of Applied Microbiology*, *134*(8), lxad161.

Sagar, N. A., Pareek, S., Benkeblia, N., & Xiao, J. (2022). Onion (*Allium cepa* L.) bioactives: Chemistry, pharmacotherapeutic functions, and industrial applications. *Food Frontiers*, *3*(3), 380-412.

Senthilkumar, M., Amaresan, N., & Sankaranarayanan, A. (2020). Quantitative Estimation of Sulfate Produced by Sulfur-Oxidizing Bacteria. In *Plant-Microbe Interactions: Laboratory Techniques* (pp. 73-75). New York, NY: Springer US.

Shah, S. H., Islam, S., & Mohammad, F. (2022). Sulphur as a dynamic mineral element for plants: a review. *Journal of Soil Science and Plant Nutrition*, *22*(2), 2118-2143.

Singh, A. L., Rani, K., Jena, R., Kona, P., Reddy, K. K., & Gangadhara, K. (2022). Microbes-based bio-stimulants towards sustainable oilseeds production: Nutrient recycling and genetics involved. In *New and Future Developments in Microbial Biotechnology and Bioengineering* (pp. 111-130). Elsevier.

Singleton, V. L., & Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

Thomloudi, E. E., Tsalgatidou, P. C., Douka, D., Spantidos, T. N., Dimou, M., Venieraki, A., & Katinakis, P. (2019). Multistrain versus single-strain plant growth promoting microbial inoculants-The compatibility issue. *Hell. Plant Prot. J*, *12*(6), 61-77.

Vidyalakshmi, R., Paranthaman, R., and Bhakyaraj, R. (2009). Sulphur Oxidizing Bacteria and Pulse Nutrition- A Review. *World Journal of Agricultural Sciences*, *5*(3), 270-278.

Vishnu, Sharma, P., Kaur, J., Gosal, S. K., & Walia, S. S. (2024). Characterization of Sulfur Oxidizing Bacteria and Their Effect on Growth Promotion of *Brassica napus* L. *Journal of Basic Microbiology*, *64*(12), e2400239.

Vishwakarma, S. K., Ilyas, T., Shahid, M., Malviya, D., Kumar, S., Singh, S., & Singh, H. V. (2024). Bacillus spp.: nature's gift to agriculture and humankind. In *Applications of bacillus and bacillus derived genera in agriculture, biotechnology and beyond* (pp. 1-36). Singapore: Springer Nature Singapore.

Wang, C., & Kuzyakov, Y. (2024). Mechanisms and implications of bacterial–fungal competition for soil resources. The ISME Journal, 18(1), wrae073.

Yebalework, W. A., Robi, N. D., Hora, D. A., Chala, E. E., & Abebe, Y. A. (2024). Nitrogen and Sulfur Fertilizer Application on Subsequent Storage Potential and Quality of Onion (*Allium cepa*) Bulb in the Central Rift Valley of Ethiopia. *Agricultural Research*, *13*(2), 300-308.

Yim, C. (2019). Organic sulfur-bearing species as subsurface carbon storage vectors. (Master's thesis, University of Calgary, Calgary, Canada).

Zenda, T., Liu, S., Dong, A., & Duan, H. (2021). Revisiting sulphur The once neglected nutrient: It's roles in plant growth, metabolism, stress tolerance and crop production. *Agriculture*, *11*(7), 626.