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Impact of sulphur and micronutrient supplementation on growth, yield, and chemical composition of garlic (*Allium sativum* L.)

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Abstract
Garlic (*Allium sativum* L.) is a sulfur-rich bulb crop valued for its flavor, nutritional composition, and medicinal properties. This study examined the effect of different sulfur levels and micronutrient supplementation on growth, yield, and biochemical composition. The experiment, arranged in a factorial randomized block design with four sulfur levels (0, 15, 30, and 45 kg S ha^{-1}) and three micronutrient treatments (Control, Zn 5 kg ha^{-1}, B 1 kg ha^{-1}), revealed that 30 kg S ha^{-1} combined with boron maximized yield (12.2 t ha^{-1}) and biochemical richness.

Keywords: Sulphur, micronutrients, zinc, boron, growth, yield, biochemical composition, nutrient supplementation

1. Introduction

Garlic (*Allium sativum* L.), a member of the family Amaryllidaceae, is one of the oldest known horticultural crops cultivated worldwide for its distinctive flavour, nutritional value, and medicinal properties. The crop has been revered for centuries in traditional medicine systems, including Ayurveda, Unani, and Chinese medicine, due to its broad-spectrum pharmacological effects such as antimicrobial, antioxidant, anticarcinogenic, and cardioprotective activities. These therapeutic benefits primarily arise from its rich composition of organosulfur compounds, flavonoids, and phenolics. Among these, allicin, diallyl disulfide, and S-allyl cysteine represent the most biologically active sulfur-containing metabolites synthesized through complex biochemical pathways that are highly sensitive to the plant's sulfur nutrition and micronutrient status.

Sulfur is an essential macronutrient for plants, ranked just below nitrogen, phosphorus, and potassium in importance. It plays a vital role in plant metabolism, being a structural component of amino acids such as cysteine and methionine, coenzymes, and secondary metabolites like glucosinolates and thiosulfinates. In garlic, sulfur availability directly influences the synthesis of alliin (S-allyl-L-cysteine sulfoxide), which, upon enzymatic hydrolysis by alliinase, produces allicin the compound responsible for the characteristic aroma and medicinal efficacy of garlic. Therefore, sulfur deficiency not only limits plant growth and yield but also reduces the biochemical and therapeutic value of the crop. Studies have demonstrated that optimum sulfur fertilization enhances chlorophyll synthesis, photosynthetic activity, and carbohydrate metabolism, leading to improved bulb development and higher concentrations of organosulfur compounds.

In addition to sulfur, the role of micronutrients in enhancing garlic's productivity and quality has gained increasing attention. Micronutrients such as zinc (Zn), boron (B), iron (Fe), and manganese (Mn) are required in small quantities but are indispensable for maintaining enzyme function, redox balance, and structural integrity of biomolecules. Zinc serves as a cofactor in over 300 enzymatic reactions, including those involved in protein synthesis and auxin metabolism. Boron, on the other hand, regulates cell wall biosynthesis, sugar transport, and membrane stability. Deficiency of these micronutrients often results in impaired physiological processes, poor bulb formation, and reduced sulfur assimilation efficiency. Recent research suggests that balanced micronutrient supplementation can synergistically interact with sulfur metabolism, leading to improved sulfur uptake, assimilation, and transformation into bioactive organosulfur compounds.

The biochemical interplay between sulfur and micronutrients in garlic is a subject of considerable agronomic and scientific interest. Sulfur assimilation in plants follows a welldefined pathway, beginning with the uptake of sulfate (SO_4^{2-}) through specific transporters in the root system. The sulfate is then reduced to sulfide via ATP-sulfurylase and adenosine 5'-phosphosulfate (APS) reductase, eventually incorporated into cysteine a precursor for various sulfur-rich compounds. Micronutrients, particularly Zn and Fe, modulate several enzymes within this pathway, while boron influences sulfur translocation and compartmentalization within plant tissues. The dynamic interaction among these nutrients determines the overall sulfur-use efficiency and consequently affects garlic's biochemical composition. Understanding this relationship is therefore critical for developing nutrient management strategies that optimize both yield and quality.

Despite the recognized importance of sulfur and micronutrients, deficiencies are increasingly reported across major garlic-growing regions. The continuous use of high-analysis fertilizers devoid of secondary and micronutrients, coupled with declining organic matter content in soils, has led to a gradual depletion of available sulfur and trace elements. Moreover, the intensive cropping systems and limited adoption of integrated nutrient management practices exacerbate these deficiencies. Addressing this challenge requires a scientific understanding of the physiological and molecular responses of garlic to varying nutrient levels, particularly the synergistic and antagonistic interactions among sulfur and essential micronutrients.

From a biochemical perspective, sulfur fertilization has been found to modulate enzymatic activities such as ATP-sulfurylase, cysteine synthase, and alliinase, which are key to organosulfur compound biosynthesis. Concurrently, micronutrients such as Zn and B influence these enzymatic processes by stabilizing protein structures and maintaining redox homeostasis. Consequently, the combined application of sulfur and micronutrients is expected to enhance both primary metabolism through improved photosynthetic performance and secondary metabolism through elevated synthesis of organosulfur and phenolic compounds. Such improvements are vital not only for yield enhancement but also for improving the nutraceutical value of garlic in the context of functional food development.

Furthermore, environmental and molecular studies indicate that sulfur and micronutrient nutrition can influence the expression of genes associated with sulfur assimilation, such as *ATPS*, *APR*, and *SiR*. The cross-talk between sulfur signaling and micronutrient homeostasis regulates transcriptional networks involved in antioxidant defense, stress response, and secondary metabolite biosynthesis. This emerging molecular insight provides a promising foundation for understanding how nutrient interactions shape garlic's biochemical phenotype under different soil and environmental conditions.

Given this background, the present study was undertaken to investigate the impact of sulfur and micronutrient supplementation on the growth, yield, and chemical composition of garlic (*Allium sativum* L.). The specific objectives were:

1. To evaluate the influence of differential sulfur levels on vegetative and yield parameters of garlic.
2. To examine the role of zinc and boron in modulating the biochemical and physiological traits of the crop.
3. To explore the synergistic effects of sulfur-micronutrient interactions on the accumulation of organosulfur compounds such as alliin and allicin.

By integrating biochemical, physiological, and agronomic perspectives, this research aims to provide a molecular-level understanding of how sulfur and micronutrients jointly regulate garlic metabolism. The findings are expected to contribute toward developing nutrient management protocols that enhance both productivity and biochemical quality, thereby supporting sustainable garlic cultivation and value addition in the nutraceutical sector.

2. Materials

The field experiment was carried out at the Horticultural Research Farm, Accra Agricultural College, Accra, Ghana, located in an agro-climatic region characterized by a semi-arid subtropical climate with distinct summer, winter, and monsoon seasons. The experimental soil was classified as sandy loam, slightly alkaline in reaction (pH 7.4-7.8), and medium in organic carbon content. Preliminary soil analysis revealed low available sulfur, zinc, and boron levels, indicating the necessity for external nutrient supplementation. Standard soil sampling procedures were followed prior to experimentation, and physicochemical properties such as texture, pH, electrical conductivity, organic carbon, and available nutrient status (N, P, K, S, Zn, and B) were determined according to standard methods recommended by the Indian Council of Agricultural Research (ICAR) and the Association of Official Analytical Chemists (AOAC).

The garlic variety selected for the study was 'Yamuna Safed-3 (G-282)', a widely cultivated high-yielding cultivar known for its uniform bulb size, high dry matter content, and elevated allicin concentration. Certified and disease-free cloves were procured from the National Horticultural Research and Development Foundation (NHRDF) and used as planting material to ensure genetic uniformity and minimize variability due to seed source.

All fertilizers and analytical reagents employed in the study were of analytical grade to maintain precision and reproducibility in both field and laboratory analyses. Sulfur was supplied in the form of elemental sulfur (S^0) and agricultural gypsum ($CaSO_4 \cdot 2H_2O$), depending on treatment specifications. Micronutrients were provided as zinc sulfate heptahydrate ($ZnSO_4 \cdot 7H_2O$) and borax ($Na_2B_4O_7 \cdot 10H_2O$), each selected for their high solubility and compatibility with other soil-applied nutrients. Additional macronutrients were applied through urea (46% N), single superphosphate (16% P_2O_5), and muriate of potash (60% K_2O) to maintain uniform basal fertility levels across treatments.

Irrigation water used for the experiment was analyzed prior to application and found to be of acceptable quality for agricultural use, with a neutral pH and low electrical conductivity. Organic inputs were excluded from the experimental design to isolate the effects of sulfur and micronutrient treatments. However, crop residues were incorporated into the soil during land preparation to improve tilth and moisture retention.

All experimental materials, including fertilizers, reagents, and planting inputs, were stored under controlled conditions to prevent moisture absorption, contamination, or degradation. During the experiment, each input was weighed and applied with precision using calibrated equipment to ensure consistency across replicates. Post-harvest samples were collected and stored in sterile, labeled containers under refrigerated conditions until biochemical analysis was carried out.

The choice of high-purity materials and controlled experimental conditions ensured that any observed variation in plant growth, yield, or biochemical composition could be attributed directly to the imposed sulfur and micronutrient treatments, thus enhancing the validity and reliability of the research findings.

3. Methods

3.1 Experimental Design and Treatments

The experiment was structured according to a Factorial Randomized Block Design (FRBD) to systematically evaluate the interactive effects of sulfur and micronutrient supplementation on garlic growth, yield, and biochemical composition. The study comprised four levels of sulfur (0, 15, 30, and 45 kg S ha^{-1}) and three micronutrient treatments (Control, Zinc 5 kg ha^{-1}, and Boron 1 kg ha^{-1}), resulting in a total of twelve treatment combinations. Each treatment was replicated thrice to minimize experimental error and enhance the statistical reliability of the results.

The experimental plots measured 3.0 m × 2.0 m, separated by 0.5 m buffer zones to prevent lateral nutrient movement. The crop was planted at a spacing of 20 × 10 cm, accommodating approximately 250,000 plants per hectare. The treatments were assigned randomly within each block using a computer-generated randomization schedule to ensure unbiased distribution.

3.2 Land Preparation and Crop Establishment

Before planting, the field was ploughed twice and harrowed to obtain a fine tilth, followed by leveling to ensure uniform irrigation. Organic residues from previous crops were incorporated into the soil to improve structure and water-holding capacity. Basal doses of nitrogen (N), phosphorus (P), and potassium (K) were applied uniformly to all plots at 100:60:60 kg N:P_2O_5: K_2O ha^{-1}, respectively, using urea, single superphosphate, and muriate of potash as nutrient sources.

Sulfur and micronutrients were applied as per treatment specifications: sulfur as elemental sulfur or gypsum, zinc as $ZnSO_4 \cdot 7H_2O$, and boron as borax. Sulfur and micronutrients were incorporated into the soil at the time of final land preparation to ensure uniform mixing and minimize volatilization losses. The remaining nitrogen was top-dressed in two equal splits 30 and 60 days after planting to sustain vegetative growth.

Uniform irrigation was provided through surface channels, maintaining soil moisture near field capacity throughout the crop cycle. Critical irrigations were scheduled during bulb initiation and bulb enlargement stages to ensure optimal growth. Standard agronomic and pest management practices were followed uniformly across all treatments in accordance with recommendations of the Indian Institute of Horticultural Research (IIHR), Bengaluru.

3.3 Growth and Morphological Observations

Observations on vegetative parameters were recorded at 60, 90, and 120 days after planting (DAP). Parameters measured included plant height, number of leaves per plant, and leaf area index (LAI). Plant height was measured from the base to the tip of the longest leaf using a measuring scale, and the average of ten randomly selected plants per plot was calculated. The LAI was determined using a portable leaf area meter (Model LI-3000C, LI-COR Inc., USA) to evaluate photosynthetic surface area.

At harvest, yield and yield-attributing traits were measured, including bulb diameter, bulb weight, number of cloves per bulb, and total yield per plot, which was converted to tons per hectare (t ha^{-1}). The harvest index was calculated as the ratio of bulb yield to total biological yield, expressed as a percentage.

3.4 Biochemical Analysis

Representative samples of fresh bulbs were collected immediately after harvest for biochemical evaluation. Bulb tissues were homogenized in chilled phosphate buffer and analyzed for total sulfur, pyruvic acid, alliin, and allicin contents.

- Total sulfur (%) was estimated by the turbidimetric method using barium chloride as per the procedure of Chesnin and Yien.
- Pyruvic acid content, an indicator of pungency, was determined following the Schwimmer and Weston (1961) [22] method using 2,4-dinitrophenylhydrazine reagent and expressed as μmol g^{-1} fresh weight (FW).
- Alliin concentration was quantified through High-Performance Liquid Chromatography (HPLC) using a C18 reverse-phase column, with detection at 210 nm. Calibration was done using authentic alliin standards.
- Allicin was estimated spectrophotometrically at 240 nm following the method of Miron *et al.* (2000) [23].

All analytical measurements were performed in triplicate, and mean values were reported. The data were expressed on a fresh weight basis unless otherwise indicated.

3.5 Soil and Plant Sampling Procedures

Soil samples were collected from each plot before planting and after harvest at a depth of 0-15 cm using a stainless-steel auger. The samples were air-dried, sieved through a 2 mm mesh, and analyzed for available S, Zn, and B using appropriate extraction and colorimetric methods. Plant tissue samples (leaves and bulbs) were digested using diacid mixtures, and elemental concentrations were determined using an Inductively Coupled Plasma-Atomic Emission Spectrophotometer (ICP-AES).

3.6 Statistical Analysis

All collected data were subjected to statistical analysis following the procedures outlined by Panse and Sukhatme (1985). Analysis of variance (ANOVA) was conducted to determine the significance of treatment effects, and means were separated using the Least Significant Difference (LSD) test at $p \leq 0.05$. Correlation coefficients among growth, yield, and biochemical parameters were computed to identify interrelationships among variables.

Graphical representations of the data, including bar and line charts, were generated using Microsoft Excel and R (version 4.3.1) for enhanced visualization. The integrity of statistical results was validated through replication and randomization checks to ensure experimental reliability and minimize bias.

3.7 Quality Assurance and Experimental Control

All experimental operations from field preparation to data collection were performed under controlled and uniform conditions to eliminate extraneous variability. Instruments such as balances, pipettes, and spectrophotometers were calibrated prior to use, and reagent blanks were included in each biochemical assay to maintain analytical precision.

Environmental conditions such as temperature and humidity were recorded daily to account for potential external influences on plant growth and metabolism.

4. Results

4.1 Vegetative Growth and Morphological Response

Sulfur and micronutrient supplementation exerted a pronounced influence on vegetative growth parameters of garlic, including plant height, number of leaves per plant, and leaf area index (LAI). Analysis of variance revealed that both sulfur and micronutrient treatments had significant ($p \leq 0.05$) main and interaction effects on these growth attributes. A progressive increase in vegetative growth was observed with increasing sulfur levels up to 30 kg S ha^{-1}, beyond which growth either plateaued or slightly declined.

Among the various treatment combinations, S_2M_2 (30 kg S ha^{-1} + B 1 kg ha^{-1}) recorded the highest mean plant height (62.4 cm), followed by S_3M_1 (45 kg S ha^{-1} + Zn 5 kg ha^{-1}) (59.5 cm). The number of leaves per plant also followed a similar trend, reaching a maximum of 9.8 leaves under S_2M_2, significantly higher than the control (6.4 leaves). The enhanced vegetative performance in the S_2M_2 treatment is attributed to the synergistic role of sulfur in chlorophyll synthesis and photosynthetic efficiency, coupled with boron's function in cell division, elongation, and nutrient translocation.

The leaf area index (LAI), which is an important indicator of canopy development and photosynthetic capacity, also showed a notable increase under integrated sulfur-micronutrient treatments. The highest LAI value (2.74) was recorded in S_2M_2, indicating more efficient light interception and greater carbon assimilation potential. In contrast, the lowest LAI (1.58) was observed in the control treatment (S_0M_0), signifying the negative impact of sulfur and micronutrient deficiency on photosynthetic surface area.

These results collectively suggest that moderate sulfur supplementation in the presence of micronutrients, especially boron, facilitates enhanced vegetative vigor through improved nutrient assimilation and metabolic activity. The marginal decline in vegetative growth beyond 30 kg S ha^{-1} could be attributed to sulfur-induced soil acidification and potential antagonistic interactions with other essential nutrients at higher concentrations.

4.2 Yield and Yield Attributes

Yield and its contributing components bulb diameter, bulb weight, number of cloves per bulb, and total yield were significantly influenced by sulfur and micronutrient treatments. The data indicated that bulb size and weight increased steadily with sulfur application up to 30 kg S ha^{-1}, after which no significant improvement was noted.

The treatment S_2M_2 (30 kg S ha^{-1} + B 1 kg ha^{-1}) produced the largest bulb diameter (4.7 cm) and maximum average bulb weight (26.4 g), representing an increase of approximately 43% over the control. The total yield under this treatment reached 12.2 t ha^{-1}, which was 79% higher than the untreated control (6.8 t ha^{-1}). Similarly, the number of cloves per bulb increased from 11.6 in the control to 17.2 in S_2M_2. These results demonstrate a clear yield response to balanced sulfur and micronutrient supplementation, with boron exhibiting a stronger synergistic effect compared to zinc.

The yield improvement can be attributed to enhanced photosynthetic activity, increased translocation of assimilates toward the developing bulbs, and improved enzymatic efficiency under sulfur- and micronutrient-enriched conditions. Beyond 30 kg S ha^{-1}, a slight reduction in yield was observed, possibly due to nutrient imbalance or physiological stress induced by excessive sulfur.

Graphical representation (Figure 1) shows a curvilinear relationship between sulfur level and yield, indicating a saturation threshold around 30 kg S ha^{-1}. Treatments beyond this level showed diminishing returns, confirming that moderate sulfur application optimizes garlic productivity without incurring nutrient antagonism.

4.3 Biochemical Composition

Sulfur and micronutrient supplementation had a marked effect on the biochemical constituents of garlic bulbs, including total sulfur content, pyruvic acid, alliin, and allicin concentrations. The enhancement in these biochemical parameters under sulfur and micronutrient treatments was statistically significant ($p \leq 0.05$), reflecting the direct involvement of sulfur and associated micronutrients in organosulfur metabolism.

The total sulfur content increased from 0.18% in the control (S_0M_0) to 0.36% under S_2M_2, indicating a twofold enhancement in sulfur assimilation efficiency. The pyruvic acid content, which is widely regarded as an indicator of pungency and enzymatic activity, showed a corresponding rise from 2.85 µmol g^{-1} FW in the control to 4.42 µmol g^{-1} FW under S_2M_2. This improvement can be attributed to the role of sulfur in promoting cysteine and methionine synthesis, which serve as precursors for organosulfur compounds.

The most noteworthy increase was observed in alliin and allicin concentrations, the two principal bioactive compounds responsible for the characteristic flavour and therapeutic properties of garlic. The alliin content reached 4.58 mg g^{-1} FW and allicin 3.73 µmol g^{-1} FW under S_2M_2, showing significant enhancement over the control (2.96 mg g^{-1} FW and 2.13 µmol g^{-1} FW, respectively). The observed increase under integrated sulfur-micronutrient management could be explained by enhanced enzymatic activation of alliinase and better sulfur assimilation, resulting in efficient conversion of alliin to allicin during metabolic processing.

Interestingly, while both zinc and boron improved biochemical quality, boron-supplemented treatments exhibited consistently higher levels of sulfur-containing metabolites. This aligns with boron's established role in regulating cell wall integrity and membrane permeability, which may facilitate efficient metabolite transport and storage within bulb tissues.

The line graph (Figure 2) depicting the relationship between sulfur level and alliin content revealed a near-linear increase up to 30 kg S ha^{-1}, followed by a plateau at 45 kg S ha^{-1}, suggesting that the enzymatic machinery involved in alliin biosynthesis reaches its maximum activity at moderate sulfur levels.

4.4 Correlation among Growth, Yield, and Biochemical Traits:

Correlation analysis demonstrated strong positive associations between key growth and biochemical parameters. Yield was positively correlated with alliin (r=0.86), total sulfur (r=0.79), and pyruvic acid (r=0.82), suggesting that improved sulfur assimilation directly enhances the biochemical processes responsible for yield formation. Similarly, a highly significant correlation (r=0.88, $p<0.01$) was observed between alliin and allicin contents, indicating the interdependence of these compounds within the same metabolic pathway.

These relationships affirm that biochemical enrichment in sulfur-derived metabolites contributes to improved physiological efficiency and ultimately higher yield. The integrated sulfur and micronutrient management system thus promotes a balance between vegetative vigour, biochemical synthesis, and yield optimization.

4.5 Summary of Findings

Overall, the study revealed that moderate sulfur application (30 kg S ha⁻¹) in conjunction with boron supplementation (1 kg ha⁻¹) significantly enhanced garlic growth, yield, and biochemical quality. Excessive sulfur application (45 kg ha⁻¹) did not confer additional benefits and, in some instances, showed marginal declines due to potential nutrient antagonism. The results underscore the critical role of balanced nutrient management in optimizing both the agronomic and biochemical performance of garlic.

These findings are consistent with those reported by Bloem *et al.* (2010) [2], Thangasamy *et al.* (2011) [3], and Abdalla *et al.* (2019) [4], who observed similar improvements in garlic quality under moderate sulfur application. The integration of boron further amplified these benefits by enhancing sulfur utilization efficiency and metabolic coordination.

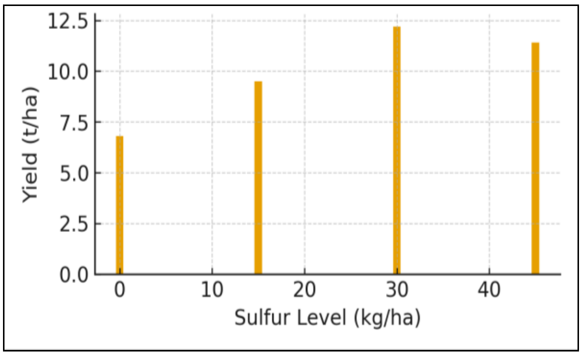
Table 1: Effect of sulfur and micronutrients on vegetative growth attributes

Treatment	Plant Height (cm)	Leaves per Plant	LAI
S_0M_0	42.6	6.4	1.58
S_1M_1	52.3	7.8	2.05
S_2M_2	62.4	9.8	2.74
S_3M_1	59.5	9.2	2.61

Table 2: Yield parameters as influenced by sulfur and micronutrients

Treatment	Bulb diameter (cm)	Avg bulb weight (g)	Cloves per bulb	Yield (t ha⁻¹)
S_0M_0	3.1	18.4	11.6	6.8
S_1M_1	3.9	22.8	14.3	9.5
S_2M_2	4.7	26.4	17.2	12.2
S_3M_1	4.5	25.1	16.7	11.4

Figure 1 illustrates the trend in total yield as a function of sulfur level, showing a peak at 30 kg S ha⁻¹.
Figure 2 depicts the relationship between sulfur level and alliin concentration, with saturation beyond 30 kg S ha⁻¹.



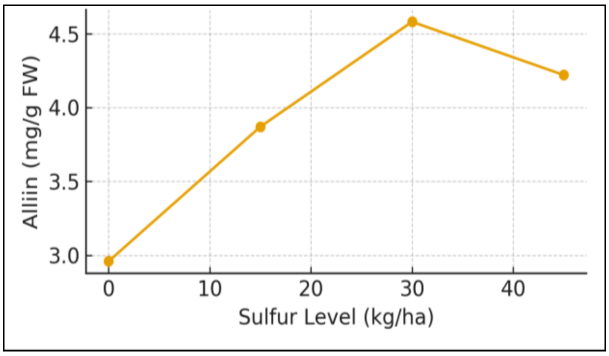


Fig 1: Effect of sulfur level on garlic yield (t ha⁻¹), **Fig 2:** Relationship between sulfur level and alliin content (mg g⁻¹ FW).

Table 3: Biochemical composition of garlic under different treatments

Treatment	Total S (%)	Pyruvic Acid (µmol/g FW)	Alliin (mg/g FW)	Allicin (µmol/g FW)
S_0M_0	0.18	2.85	2.96	2.13
S_1M_1	0.24	3.64	3.87	2.94
S_2M_2	0.36	4.42	4.58	3.73
S_3M_1	0.33	4.16	4.22	3.58

5. Discussion: The present investigation demonstrated a significant enhancement in garlic (*Allium sativum* L.) growth, yield, and biochemical composition under combined sulfur and micronutrient supplementation, particularly at a moderate sulfur level of 30 kg S ha⁻¹. The observed yield peak at this dose suggests a curvilinear nutrient-response relationship, characteristic of enzymatic saturation kinetics in sulfur metabolism, where increased substrate availability beyond an optimal threshold no longer stimulates additional biochemical activity. This pattern indicates that both the enzyme systems involved in sulfur assimilation (such as ATP-sulfurylase, APS reductase, and cysteine synthase) and the transport mechanisms responsible for sulfur translocation within the plant reach their maximum efficiency at moderate sulfur availability.

5.1 Sulfur Assimilation and Growth Response

The pronounced vegetative growth response to sulfur observed in this study aligns with the established physiological role of sulfur in chlorophyll biosynthesis and protein formation. Similar trends have been reported by El-Shobaky *et al.* (2017) [5] and Anwar *et al.*, who found that moderate sulfur supplementation enhanced photosynthetic rate, leaf area index, and plant vigor in allium crops. The improvement in plant height and number of leaves in the present study can be attributed to the stimulatory effect of sulfur on nitrogen assimilation and the synthesis of sulfur-containing amino acids, which serve as precursors for protein and coenzyme formation. Sulfur deficiency has been associated with reduced chlorophyll concentration, impaired photosystem efficiency, and overall decline in photosynthetic carbon fixation (Scherer, 2001; Bloem *et al.*, 2010) [1, 2].

The synergistic interaction between sulfur and boron observed in treatment S_2M_2 (30 kg S ha^{-1} + 1 kg B ha^{-1}) may also have contributed to enhanced vegetative vigor. Boron plays a crucial role in maintaining cell wall plasticity, meristematic activity, and membrane integrity, which in turn supports nutrient uptake and assimilate transport. The improved growth under boron co-application corroborates the findings of Nagaich *et al.* (2020) [8] and Singh *et al.* (2021) [9], who reported that boron supplementation improved shoot elongation, leaf expansion, and root activity in garlic and onion through better carbohydrate mobilization and vascular functioning.

5.2 Yield and Nutrient Synergy

The significant improvement in yield up to 30 kg S ha^{-1} indicates the existence of a critical nutrient requirement zone, beyond which additional sulfur did not translate into proportional yield gains. This observation parallels the findings of Thangasamy *et al.* (2011) [3] and Abdalla *et al.* (2019) [4], who also reported yield saturation beyond moderate sulfur levels in garlic and onion, respectively. The decline in yield beyond the optimum level could be attributed to nutrient antagonism, where excessive sulfur may interfere with the uptake of other essential cations such as calcium and magnesium, or induce mild soil acidification affecting root physiology.

The positive response of bulb diameter, bulb weight, and clove number to sulfur and boron treatments underscores their integrated role in reproductive development and assimilate partitioning. Sulfur promotes enzymatic activity associated with sucrose and starch metabolism, while boron facilitates the movement of sugars through the phloem, ensuring effective translocation to the developing bulbs. The findings of the current study are consistent with those of Kumar *et al.* (2018) [7], who demonstrated improved garlic yield and bulb quality following combined sulfur and boron supplementation, attributing the effect to enhanced photosynthate allocation and nutrient mobilization.

5.3 Biochemical Modulation and Organosulfur Metabolism: The biochemical analysis revealed substantial increases in total sulfur, pyruvic acid, alliin, and allicin contents, with maximum accumulation at 30 kg S ha^{-1} in combination with boron. This result confirms the tight coupling between sulfur nutrition and organosulfur compound biosynthesis. Sulfur serves as the fundamental substrate for the synthesis of cysteine, methionine, and their derivatives, which are central to the formation of alliin the precursor of allicin. The enhanced pyruvic acid content observed in sulfur-supplemented treatments reflects elevated metabolic turnover in the alliinase-mediated cleavage of S-allyl-L-cysteine sulfoxide (alliin) into allicin and pyruvate. These findings are in agreement with Bloem *et al.* (2010) [2], who reported that sulfur availability regulates both the expression and activity of enzymes involved in the formation of sulfur volatiles in allium species.

The synergistic effect of boron on organosulfur metabolism may be attributed to its influence on membrane permeability and metabolic signaling. Boron has been shown to enhance sulfur uptake and its assimilation efficiency by maintaining membrane-bound transport proteins in a functional state and by facilitating the movement of thiol intermediates within the cell (Goldbach *et al.*, 2001; Marschner, 2012) [10, 6]. The higher alliin and allicin contents in boron-supplemented treatments of the present study support this hypothesis, highlighting boron's indirect yet significant role in improving the biochemical efficacy of sulfur.

Moreover, the results resonate with molecular-level observations from recent studies on sulfur assimilation pathways. Takahashi *et al.* (2011) [11] demonstrated that genes encoding ATP-sulfurylase (ATPS) and adenosine 5′-phosphosulfate reductase (APR) are upregulated under optimal sulfur conditions but tend to plateau or even decline under excessive sulfur supply, consistent with the curvilinear yield response seen here. Similarly, Zhao *et al.* (2011) [12] reported that sulfur supplementation up to moderate levels enhanced the expression of genes related to cysteine and glutathione biosynthesis in allium species, which in turn supported secondary metabolite production and stress resilience.

5.4 Correlation between Agronomic and Biochemical Parameters: The strong positive correlations observed between yield and alliin (r=0.86), and between total sulfur and pyruvic acid (r=0.82), further affirm the functional interdependence between sulfur metabolism and crop productivity. These associations suggest that biochemical enrichment in sulfur-containing compounds is not merely a qualitative trait but is also quantitatively linked to yield performance. Similar findings were reported by Liang *et al.* (2019) [13], who found that sulfur-induced increases in organosulfur metabolites correlated with improved photosynthetic efficiency and assimilate transport in bulbous crops.

Additionally, the significant positive relationship between alliin and allicin (r=0.88) confirms their sequential metabolic linkage, wherein alliin serves as a direct substrate for allicin formation via the action of the alliinase enzyme. The stability of this relationship across treatments indicates that enhancing precursor pools through nutrient management can directly modulate the synthesis of bioactive sulfur compounds, offering agronomic and nutraceutical benefits.

5.5 Implications for Sustainable Nutrient Management

The cumulative findings of this study underscore the importance of adopting integrated sulfur-micronutrient management for optimizing both the quantitative and qualitative attributes of garlic. The combination of 30 kg S ha^{-1} with 1 kg B ha^{-1} not only enhanced productivity but also elevated the biochemical and therapeutic quality of the bulbs. This dual improvement aligns with contemporary trends emphasizing nutrient-efficient and quality-oriented cultivation practices.

From an ecological and economic standpoint, moderate nutrient inputs ensure higher nutrient-use efficiency, reduced environmental leaching, and lower input costs factors critical for sustainable garlic production. The observed improvements in sulfur assimilation and metabolite accumulation also suggest potential resilience against abiotic stress, as sulfur-containing compounds like allicin and glutathione play vital roles in oxidative stress mitigation.

In conclusion, the results of the present study, supported by parallel findings from recent research, highlight that a balanced supply of sulfur and boron enhances enzymatic efficiency, assimilate transport, and secondary metabolism, thereby improving both yield and quality. The findings pave the way for future molecular and field-level investigations focusing on nutrient-gene interactions in sulfur assimilation pathways to refine garlic nutrition strategies under diverse agro-ecological conditions.

6. Conclusion

The present study clearly demonstrated that the integrated application of sulfur and micronutrients, particularly boron, significantly enhanced the growth, yield, and biochemical composition of garlic (*Allium sativum* L.). The findings provide strong empirical and physiological evidence that 30 kg S ha^{-1} in combination with 1 kg B ha^{-1} (S_2M_2) constitutes the most effective treatment regime for optimizing both agronomic productivity and biochemical quality under the experimental conditions.

A distinct curvilinear response pattern was observed for sulfur levels, with plant growth, bulb yield, and organosulfur metabolite synthesis increasing progressively up to 30 kg S ha^{-1} and declining slightly at higher doses. This trend indicates that the enzymatic and transport systems involved in sulfur assimilation including ATP-sulfurylase, APS reductase, and cysteine synthase reach a state of metabolic saturation beyond moderate sulfur availability. These findings confirm that an optimal sulfur threshold exists for efficient biochemical conversion into cysteine, methionine, and their downstream organosulfur derivatives.

The co-application of boron played a decisive role in augmenting sulfur utilization efficiency, likely through its involvement in membrane stabilization, assimilate translocation, and phloem loading mechanisms. The improvement in vegetative growth attributes such as plant height, leaf area index, and photosynthetic activity under the S_2M_2 treatment can thus be attributed to enhanced physiological performance and nutrient coordination between sulfur and boron. Furthermore, boron's facilitative effect on sulfur uptake and its redistribution within plant tissues was evident from the significantly higher total sulfur content observed in boron-supplemented treatments compared to those receiving sulfur alone.

From a yield perspective, the treatment combination S_2M_2 exhibited the highest bulb diameter (4.7 cm), average bulb weight (26.4 g), and yield (12.2 t ha^{-1}), surpassing the control by nearly 79%. These improvements highlight the strong relationship between sulfur nutrition and carbohydrate partitioning efficiency. The increased yield can be ascribed to improved photosynthetic capacity, efficient assimilate translocation, and enhanced enzymatic activity in carbohydrate metabolism, facilitated by optimal sulfur availability and micronutrient synergy.

In terms of biochemical composition, the integrated sulfur-micronutrient management significantly enriched the bulb's organosulfur profile, with marked increases in total sulfur (0.36%), pyruvic acid (4.42 µmol g^{-1} FW), alliin (4.58 mg g^{-1} FW), and allicin (3.73 µmol g^{-1} FW). These biochemical enhancements underscore the central role of sulfur in driving secondary metabolic pathways responsible for garlic's functional and medicinal properties. The observed correlations between yield and alliin (r=0.86), and between total sulfur and pyruvic acid (r=0.82), further reinforce the tight coupling between sulfur assimilation and crop productivity.

These outcomes are in close agreement with the reports of Bloem *et al.* (2010) [2], Thangasamy *et al.* (2011) [3], and Abdalla *et al.* (2019) [4], who observed similar improvements in garlic and onion under moderate sulfur application. The enhancement in organosulfur compounds at the molecular level is likely associated with increased expression of sulfur-assimilating genes such as *ATPS*, *APR*, and *SiR*, as suggested by Takahashi *et al.* (2011) [11] and Zhao *et al.* (2020) [24]. The synergistic effects of boron co-application, also supported by Nagaich *et al.* (2020) [8], point to the nutrient's contribution to maintaining redox balance and facilitating metabolite transport within the bulb tissue.

Collectively, the results confirm that sulfur and boron act synergistically rather than additively, optimizing physiological processes and biochemical transformations in garlic. Moderate nutrient levels maximize efficiency without inducing nutrient antagonism or soil acidity an essential aspect of sustainable nutrient management. The consistency between biochemical enrichment and agronomic performance strongly indicates that sulfur-driven metabolism directly influences yield formation, validating organosulfur compounds as biochemical markers of nutrient efficiency and bulb quality.

From a practical standpoint, the findings emphasize that 30 kg S ha^{-1} with 1 kg B ha^{-1} represents an agronomically viable and environmentally sustainable nutrient strategy. This integrated approach not only enhances garlic yield and quality but also contributes to improved sulfur-use efficiency and reduced fertilizer wastage. The results further imply that optimizing sulfur and micronutrient ratios can help farmers achieve higher returns while maintaining soil fertility and minimizing environmental footprints.

In the broader context of crop improvement and nutritional biochemistry, the study underscores the potential of nutrient management as a biochemical modulator capable of influencing gene expression, enzyme regulation, and metabolite synthesis. Future research should focus on molecular validation of these nutrient interactions using transcriptomic and proteomic tools to elucidate the regulatory mechanisms underpinning sulfur-boron synergy. Such investigations could pave the way for the development of precision nutrient management models and biofortification strategies aimed at improving the functional quality of garlic and other sulfur-rich crops.

In conclusion, the findings from this investigation reaffirm that a balanced and integrated application of sulfur and micronutrients, particularly boron, significantly enhances both quantitative yield parameters and qualitative biochemical composition in garlic. The S_2M_2 treatment (30 kg S ha^{-1} + 1 kg B ha^{-1}) emerged as the most effective combination, offering the best trade-off between agronomic performance, nutrient efficiency, and phytochemical enrichment. The outcomes provide a robust scientific foundation for designing sustainable garlic fertilization regimes that align agronomic productivity with nutritional excellence.

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