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Effects of Chemical and Organic Fertilizers on Physiological and Biochemical Traits of Garlic (*Allium sativum* L.)

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ABSTRACT

This study evaluated the effects of chemical, organic, and biofertilizer treatments on the physiological, biochemical, antioxidant, and mineral characteristics of garlic (*Allium sativum* L.). The experiment was conducted at the Botany Department, University of Education, Faisalabad Campus, using a factorial arrangement in a completely randomized design (CRD). Twenty-five pots containing 10 kg of soil each were subjected to five treatments: T1, control (water only); T2, vermicompost (5 g

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pot^{-1}); T3, Nitrophos (5 g pot^{-1}); T4, oven-dry weight content (OWDC, 5 g pot^{-1}); and T5, kitchen-waste fertilizer (5 g pot^{-1}). The evaluated parameters included chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, total soluble protein (TSP), total soluble sugars (TSS), proline, ascorbic acid, catalase (CAT), peroxidase (POD), relative water content (RWC), net photosynthetic rate (Pn), transpiration, carbon-related parameters, and leaf N, P, and K concentrations. Nitrophos produced the highest values for chlorophyll a (0.782 mg g^{-1}), chlorophyll b (0.640 mg g^{-1}), total chlorophyll (1.422 mg g^{-1}), total soluble protein (6.40 mg g^{-1}), net photosynthetic rate (15.57 µmol m^{-2} s^{-1}), RWC (72.50%), and leaf N, P, and K (4.43, 5.80, and 41.13 mg g^{-1}, respectively). Organic treatments produced higher levels of several biochemical and antioxidant-related parameters, with OWDC showing the highest proline (0.639 mg g^{-1}), while kitchen-waste fertilizer produced the highest total soluble sugars (6.33 mg g^{-1}), ascorbic acid (76.53 mg g^{-1}), and catalase activity (159.97 U mg^{-1} protein). Nitrophos also recorded the highest peroxidase activity (144.77 U mg^{-1} protein). Overall, chemical fertilizer was most effective for improving photosynthetic performance, soluble protein, and mineral accumulation, whereas organic fertilizers enhanced several biochemical and antioxidant traits. The findings indicate that combining chemical, organic, and biological nutrient sources may provide a more balanced approach for sustainable garlic production and soil health.

INTRODUCTION

Garlic (*Allium sativum* L.), a member of the family Alliaceae, is an important bulb crop cultivated widely for culinary, nutritional, and medicinal purposes. It has been an integral component of traditional agricultural systems and is commonly used as a condiment, spice, and vegetable. Garlic contains carbohydrates, proteins, essential oils, mineral elements, vitamin C, and sulfur-containing compounds that contribute to its nutritional and health-promoting properties. Its leaves are also a valuable source of ascorbic acid, while bioactive compounds such as allicin are associated with several biological and therapeutic properties, including antioxidant and antimicrobial activities (Alizad et al., 2018; Assefa et al., 2015; Zaghloul et al., 2016; Al-Shammary & Alwan, 2015; Sharma et al., 2021; Lana et al., 2022; Gupta et al., 2024; Taha et al., 2025).

Increasing demand for garlic production requires effective nutrient management to maintain plant growth, physiological performance, and product quality while preserving soil health. Fertilization is therefore an important component of garlic production because adequate nutrient availability supports photosynthesis, protein synthesis, mineral accumulation, and other physiological processes. Previous studies have shown that both inorganic and organic fertilizers can influence garlic growth, yield, and physiological characteristics, indicating that fertilizer source is an important factor in determining crop performance (Assefa et al., 2015; Alizad et al., 2018; Rai & Negi, 2021).

Chemical fertilizers provide readily available nutrients and can consequently produce rapid improvements in plant physiological performance and nutrient uptake. However, excessive or prolonged dependence on chemical fertilizers may adversely affect soil fertility, soil microbial activity, groundwater quality, and ecosystem health. Continued

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use of chemical fertilizers without appropriate nutrient management may therefore create challenges for maintaining soil quality and sustainable crop production (Ahmed & Sarhan, 2024; Kumari, 2024). These limitations have increased interest in alternative nutrient sources that can support crop productivity while reducing potential environmental impacts.

Organic fertilizers and biofertilizers offer potential alternatives or complementary sources of plant nutrients. Organic amendments can improve soil structure, nutrient availability, and biological activity, while biofertilizers may enhance nutrient mobilization and plant growth through beneficial biological processes. Previous research has reported beneficial effects of organic amendments, including vermicompost and farmyard manure, on soil properties and nutrient uptake. Similarly, biological inputs such as arbuscular mycorrhizal fungi and *Azotobacter* have been associated with improved root development, nutrient mobilization, and plant vigor (Nditasari et al., 2023; Tajamul, 2021; Kumawat et al., 2024). The combined use of chemical, organic, and biological nutrient sources has consequently emerged as a potential strategy for maintaining crop productivity while improving the sustainability of nutrient management (Balaji, 2023).

Despite these findings, the relative effects of different fertilizer sources on the integrated physiological, biochemical, antioxidant, and mineral responses of garlic remain insufficiently characterized under the experimental conditions of the present study. In particular, there is a need to compare readily available chemical fertilizer with organic materials and biofertilizer using a common experimental framework and multiple plant-response indicators. Such a comparison can help clarify whether fertilizer sources differ in their effects on photosynthetic performance, biochemical quality, antioxidant defense, and nutrient accumulation.

Therefore, the present study aimed to compare the effects of chemical fertilizer (Nitrophos), biofertilizer (vermicompost), and organic fertilizer sources (oven-dry weight content [OWDC: It is the amount or proportion of dry plant material remaining after removing all moisture by oven drying to a constant weight.] and kitchen-waste fertilizer) on the physiological, biochemical, antioxidant, and mineral characteristics of garlic (*Allium sativum* L.). The study hypothesized that fertilizer source would differentially influence these traits, with chemical fertilizer expected to promote rapid physiological and mineral responses, whereas organic and biofertilizer treatments would provide greater benefits for biochemical and antioxidant characteristics.

MATERIALS AND METHODS

Experimental Site and Plant Material

This study was designed to investigate the effect of different type of fertilizer on the growth and physiological responses of garlic (*Allium sativum* L.) at Botany Department, University of Education, Faisalabad campus. Healthy China White cloves, which were purchased from Ayub Agriculture research institute (AARI), Faisalabad were used as planting material.

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Experimental Design and Treatments

This was design using the factorial arrangement in the CRD design. Ten kilograms of well washed soil was placed into each of the 25 pots. The uniformly sized cloves were placed into each pot.

There were five treatments which were including control group:

- **T1 (Control): Water only**
- **T2: Vermicompost (5 g pot^{-1})**
- **T3: Nitrophos (5 g pot^{-1})**
- **T4: OWDC (5 g pot^{-1})**
- **T5: Kitchen-waste fertilizer (5 g pot^{-1})**

Fertilizers were applied directly to the soil in three equal split doses during the early vegetative growth stage.

Biochemical and Physiological Measurements

Photosynthetic Pigments

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids were estimated by the methods developed by Arnon (1949) and Davies (1976). Harvested leaves were directly extracted by storing in 80% acetone over night. These extracts after being centrifuged at 14000g for 5 minutes, absorbances were recorded at 480nm, 645nm and 663nm with the help of Hitachi U2800 spectrophotometer and pigment contents were calculated with the help of standard equations.

- Determination of the protein content the protein content was measured by the procedure of Bradford (1976). Leaf extracts was mixed with Coomassie Brilliant Blue G-250 and the absorbance was recorded using standard curve with bovine serum albumin (BSA) as a reference.
- To estimate the total soluble sugars the anthrone method developed by Palma et al., 2009, was used. 0.17 g fresh leaf material was hydrolysed in distilled water at 95 o C for 1.5 hours. After addition of sulfuric acid and anthrone reagent a blue-green chromophore was formed. Absorbance was measured at 630 nm.
- Proline was determined following the methodology of Bates et al., (1973). The homogenized plant tissue was made in 3% sulfosalicylic acid, which was then centrifuged for ten minutes at 10,000 g. The supernatant was mixed with Ninhydrin solution and glacial acetic acid, and the absorbance was read at 520 nm.
- Ascorbic Acid level was estimated by titration with 2,6-dichlorophenolindophenol (DCPIP) using oxalic acid as indicator when color was not visible.
- Catalytic activity was determined using Chance and Maehly's method (1955). Enzyme extracts are incubated in 50mM phosphate buffer (pH 7.0), and H2O2 decomposition is monitored with a manometric device.
- Peroxidase reaction mixture consists of 20 mM phosphate buffer, pH 7.0, 0.05% guaiacol, 2 mM H_2O and enzyme extract were pipetted into tube. Absorbance

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reading at 470 nm was recorder for 3 minutes. Activity was calculated using extinction coefficient of 26.6 mM cm (Chance and Maehly, 1955).

RESULTS

Table 1: Effect of biofertilizer, chemical fertilizer, organic fertilizer and OWDC on different parameters of the plant.

Descriptive Statistics by Treatment (Mean ± SD)									
Variable	**T1**	**T2**	**T3**	**T4**	**T5**	**Overall**	**Minimum**	**Maximum**	**CV (%)**
Chla (mg g^{-1})	0.536 ± 0.019	0.638 ± 0.025	0.782 ± 0.009	0.626 ± 0.026	0.657 ± 0.019	0.648 ± 0.083	0.515	0.788	12.87
Chlb (mg g^{-1})	0.375 ± 0.017	0.408 ± 0.037	0.640 ± 0.014	0.515 ± 0.016	0.484 ± 0.004	0.484 ± 0.097	0.356	0.656	**20.12**
Tchl (mg g^{-1})	0.912 ± 0.025	1.045 ± 0.061	1.422 ± 0.019	1.140 ± 0.038	1.141 ± 0.023	1.132 ± 0.176	0.897	1.441	15.55
Car (mg g^{-1})	0.718 ± 0.016	0.832 ± 0.020	0.915 ± 0.016	0.816 ± 0.014	0.858 ± 0.018	0.828 ± 0.068	0.700	0.932	8.24
TSP (mg g^{-1})	4.37 ± 0.11	5.30 ± 0.26	6.40 ± 0.17	4.80 ± 0.36	5.43 ± 0.15	5.26 ± 0.73	4.300	6.500	13.93
TSS (mg g^{-1})	5.20 ± 0.10	5.17 ± 0.21	5.40 ± 0.20	6.33 ± 0.15	6.23 ± 0.21	5.67 ± 0.55	5.000	6.500	9.70
Pro (mg g^{-1})	0.534 ± 0.015	0.531 ± 0.021	0.538 ± 0.014	0.639 ± 0.031	0.611 ± 0.018	0.570 ± 0.050	0.510	0.672	8.77
AsA (umol g^{-1})	72.30 ± 2.54	73.43 ± 2.69	75.90 ± 0.36	74.43 ± 1.07	76.53 ± 0.58	74.52 ± 2.18	70.500	77.200	2.93
CAT (U mg^{-1})	154.60 ± 2.12	158.30 ± 1.85	156.87 ± 3.79	159.97 ± 1.97	156.07 ± 5.22	157.16 ± 3.36	150.200	162.200	2.14
POD	135.	141.	144.	143.	141.	141.4	132.500	147.200	2.96

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(U mg^{-1})	30 ± 4.26	73 ± 1.48	77 ± 1.12	97 ± 2.93	53 ± 3.06	6 ± 4.18			
RWC (%)	65.37 ± 0.51	63.93 ± 1.40	72.50 ± 0.89	71.23 ± 1.25	67.83 ± 0.47	68.17 ± 3.50	62.800	73.200	5.14
Pn (µmol m^{-2} s^{-1})	12.63 ± 0.15	13.63 ± 0.15	15.57 ± 0.25	13.67 ± 0.42	14.37 ± 0.12	13.97 ± 1.02	12.500	15.800	7.33
E (mmol m^{-2} s^{-1})	4.37 ± 0.12	4.87 ± 0.06	5.67 ± 0.12	5.27 ± 0.25	5.63 ± 0.29	5.16 ± 0.53	4.300	5.800	10.33
C (mg g^{-1})	0.537 ± 0.021	0.630 ± 0.020	0.710 ± 0.066	0.733 ± 0.015	0.780 ± 0.010	0.678 ± 0.093	0.520	0.790	13.73
N (%)	1.23 ± 0.03	1.45 ± 0.06	4.43 ± 0.12	1.32 ± 0.03	1.35 ± 0.05	1.96 ± 1.29	1.200	4.500	**65.71**
P (mg g^{-1})	2.47 ± 0.06	3.10 ± 0.10	5.80 ± 0.10	3.47 ± 0.15	3.53 ± 0.31	3.67 ± 1.18	2.400	5.900	**32.04**
K (mg g^{-1})	19.30 ± 0.85	31.00 ± 1.00	41.13 ± 1.01	33.87 ± 1.81	35.67 ± 2.50	32.19 ± 7.62	18.500	42.200	**23.66**

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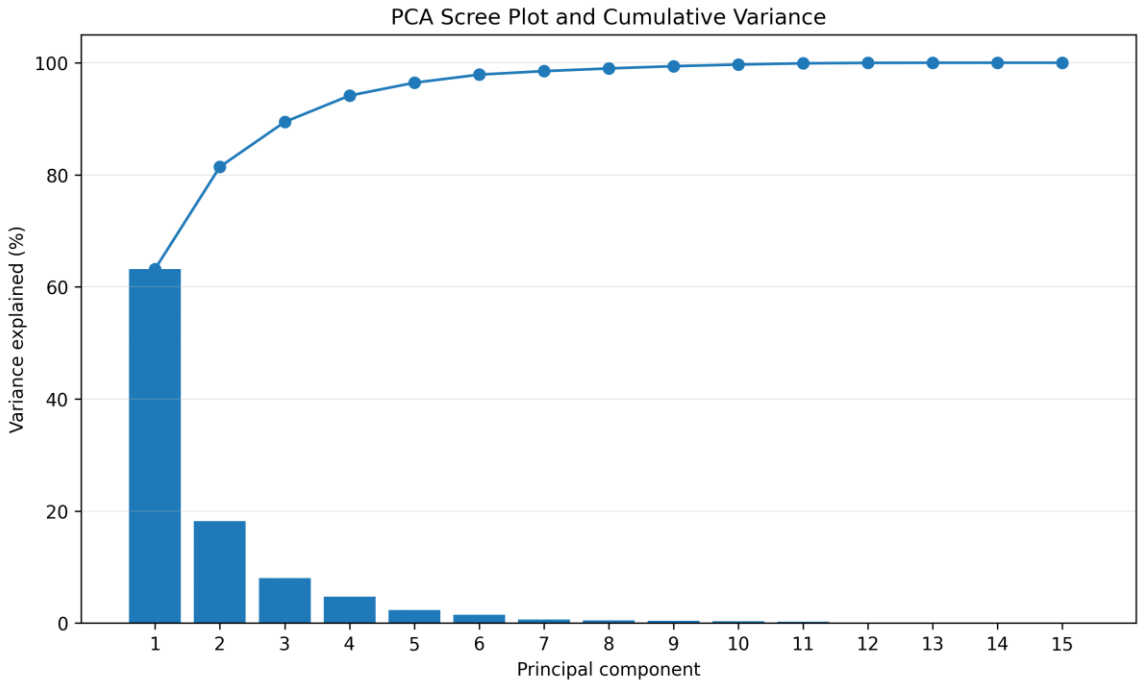

Figure 1. Principal component analysis (PCA) scree plot showing the percentage of variance explained by individual principal components and the cumulative variance explained by the principal components for growth and physiological attributes of garlic (*Allium sativum* L.) under different fertilizer treatments.

Interpretation:

Based on the screen plot analysis, 63.20% of the total variance was accounted for by the first principal component (PC1), while 18.22% by the second principal component (PC2). This implies that 81.42% of the total variance was accounted for by PC1 and PC2 together. Thus, from the data, we can conclude that the first two principle components captured most of the variation in fertilizer treatment and garlic traits. The multivariate response of garlic to fertilizer treatment is well characterized by PC1 and PC2.

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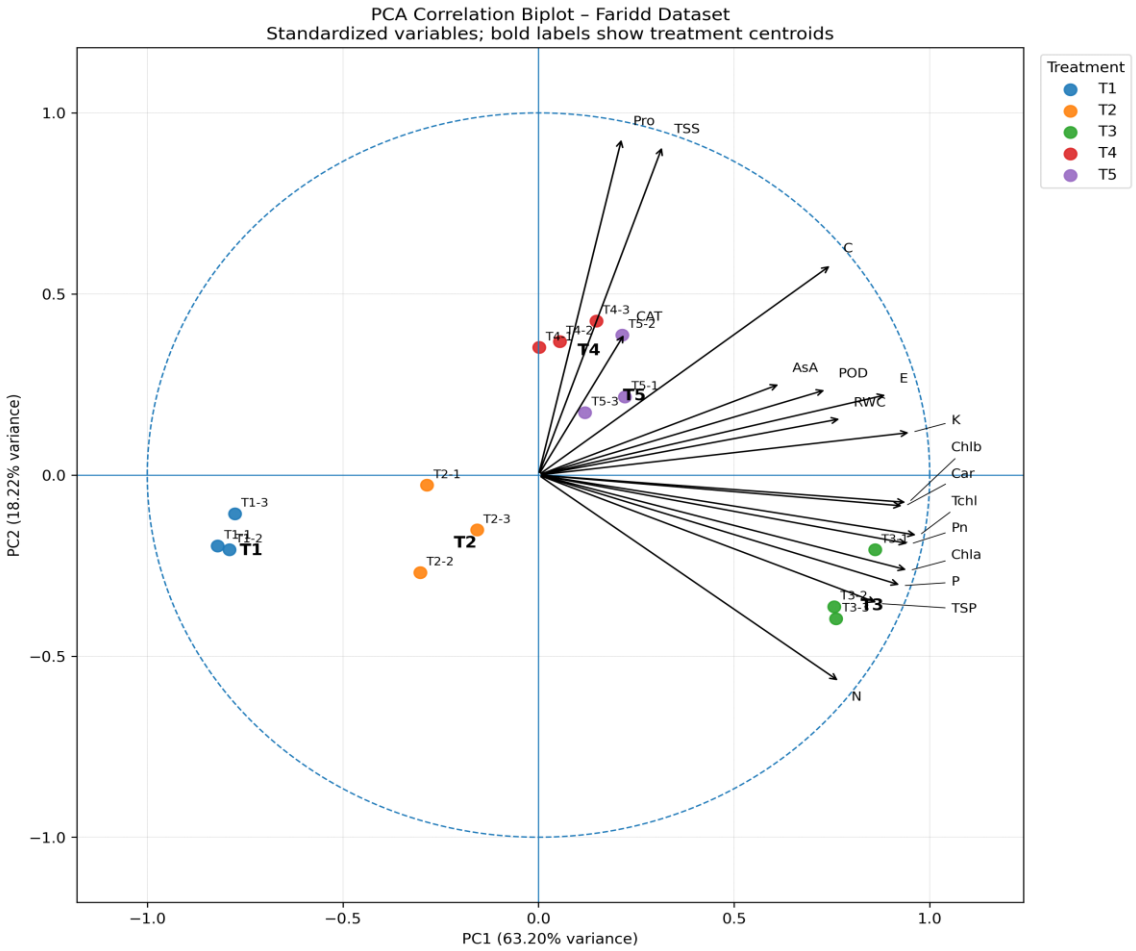

Figure 2. Principal component analysis (PCA) correlation biplot of standardized growth, physiological, biochemical, and nutrient attributes of garlic (*Allium sativum* L.) subjected to different fertilizer treatments. PC1 and PC2 explained **63.20% and 18.22%** of the total variance, respectively. Arrows represent the direction and magnitude of variable loadings, while individual points represent treatment replicates and bold labels indicate treatment centroids.

Interpretation:

As depicted in the PCA biplot, the treatments are distinctly separated multivariate. Chlorophyll content, total chlorophyll content, carotenoids, phosphorus (P), potassium (K), nitrogen (N), calcium (Ca), and other physiological parameters were some of the growth and nutritional parameters that the first principal component (PC1) largely distinguished the treatments by. The positively correlated parameters were depicted as those having arrows pointing in the same direction, while those pointing in the opposite direction were depicted as having negative correlations. It was clear that the growth, physiological, and nutritional characteristics of garlic were reacting differently to the various fertilizers used. There are some parameters whose positions are very close, which means stronger positive relationships among those traits, while their distance from particular treatment centroids reflects weaker or contrasting treatment

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associations.

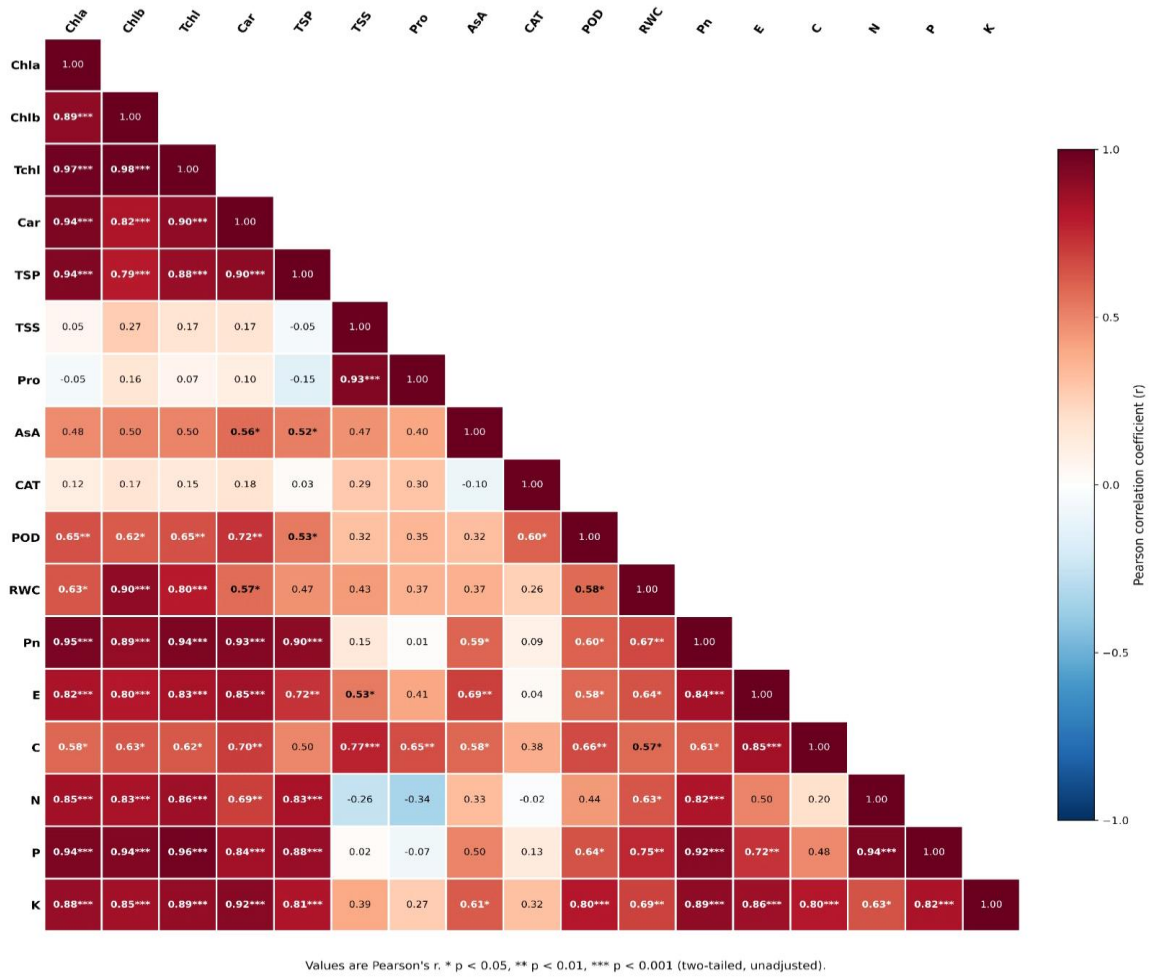

Figure 3. Pearson correlation heatmap showing the relationships among growth, physiological, biochemical, and nutrient attributes of garlic (*Allium sativum* L.). The values represent Pearson's correlation coefficient (*r*), with the color intensity indicating the strength and direction of the associations. Asterisks indicate statistically significant correlations ($P < 0.05$, $*P < 0.01$, $**P < 0.001$).

Interpretation:

There were many growth, physiological, and nutritional parameters of garlic exhibiting highly positive correlations in the correlation analysis. There were strong positive correlations among phosphorus, potassium, nitrogen, total soluble proteins, carotenoids, chlorophyll parameters, and other nutritional and physiological parameters measured in the experiment. Such correlations indicate that good nutrient supply owing to appropriate fertilization may lead to more plant growth and physiological processes. Characteristics which do not exhibit any correlation or even negative correlation suggest that there is almost no response of variables to each other. Thus, correlation analysis has confirmed the findings obtained during PCA.

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DISCUSSION

The present study showed that fertilizer source influenced the physiological, biochemical, antioxidant, and mineral characteristics of garlic (*Allium sativum* L.). The responses varied among treatments, with Nitrophos generally producing the highest values for photosynthetic pigments, net photosynthetic rate, total soluble protein, relative water content, and N, P, and K concentrations. In contrast, organic treatments, particularly OWDC and kitchen-waste fertilizer, produced higher values for several biochemical and antioxidant-related parameters, including total soluble sugars, proline, ascorbic acid, and catalase activity. Vermicompost showed comparatively intermediate responses for most of the measured traits. These findings indicate that no single fertilizer source was superior for all parameters.

Photosynthetic Pigments and Photosynthetic Performance

Nitrophos produced the highest concentrations of chlorophyll a (0.782 mg g^{-1}), chlorophyll b (0.640 mg g^{-1}), total chlorophyll (1.422 mg g^{-1}), and carotenoids (0.915 mg g^{-1}). It also resulted in the highest net photosynthetic rate (15.57 µmol m^{-2} s^{-1}) and relative water content (72.50%). The higher N concentration under Nitrophos (4.43 mg g^{-1}), together with the greater chlorophyll and photosynthetic values, is consistent with the important role of nitrogen in chlorophyll and protein formation and in maintaining photosynthetic capacity. However, because the present study did not directly investigate nutrient-release mechanisms or physiological pathways, the observed association should not be interpreted as proof of a specific causal mechanism.

The superior performance of Nitrophos for photosynthetic traits is consistent with previous studies reporting beneficial effects of inorganic fertilization on garlic growth and physiological characteristics. Assefa et al. (2015) reported positive effects of both inorganic and organic fertilizers on garlic growth and productivity, while Alizad et al. (2018) reported that fertilizer type affected garlic growth and physiological characteristics. The present findings extend these observations by showing that, under the conditions of this experiment, Nitrophos produced the highest values for several photosynthetic parameters.

Total Soluble Protein and Mineral Accumulation

Nitrophos also produced the highest total soluble protein content (6.40 mg g^{-1}) and the highest concentrations of N (4.43 mg g^{-1}), P (5.80 mg g^{-1}), and K (41.13 mg g^{-1}). In comparison, the control treatment recorded 1.23, 2.47, and 19.30 mg g^{-1} for N, P, and K, respectively. The higher nutrient concentrations were accompanied by higher chlorophyll, photosynthetic rate, and protein values under Nitrophos.

The relatively strong response to Nitrophos may be related to the availability of mineral nutrients supplied by this treatment, particularly because the treatment was associated with greater nutrient accumulation in the leaves. Nitrogen is an important component of proteins and photosynthetic machinery, while phosphorus and potassium participate in several processes associated with plant metabolism and growth. Nevertheless, the present experiment measured final nutrient concentrations rather than nutrient uptake or nutrient-release kinetics; therefore, the results demonstrate an association between Nitrophos application and greater tissue nutrient concentrations rather than establishing the underlying mechanism.

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These results are broadly consistent with previous research. Assefa et al. (2015) reported positive effects of inorganic and organic fertilizers on garlic growth and yield, while Sharma et al. (2021) also reported effects of organic and inorganic fertilizers on garlic growth, yield, and economic performance. Thus, the present study supports the potential of readily available mineral fertilization to improve physiological performance and mineral status of garlic under the experimental conditions used.

Soluble Sugars and Proline

In contrast to the photosynthetic and mineral traits, organic fertilizer treatments produced relatively higher values for several biochemical parameters. OWDC produced the highest proline concentration (0.639 mg g^{-1}), followed by kitchen-waste fertilizer (0.611 mg g^{-1}), whereas kitchen-waste fertilizer produced the highest total soluble sugar content (6.33 mg g^{-1}). Proline and soluble sugars are associated with osmotic regulation and metabolic adjustment in plants; therefore, their higher concentrations indicate differences in biochemical responses among fertilizer sources.

However, the present study did not directly measure stress conditions, osmotic potential, or the biochemical pathways responsible for metabolite accumulation. Therefore, the higher proline and soluble sugar values should be interpreted as treatment-associated biochemical responses rather than definitive evidence that organic fertilizers increased stress tolerance. The findings nevertheless indicate that the effects of fertilizer source extended beyond photosynthetic pigments and mineral accumulation.

Ascorbic Acid and Antioxidant Enzyme Activity

Kitchen-waste fertilizer produced the highest ascorbic acid concentration (76.53 mg g^{-1}), compared with 72.30 mg g^{-1} in the control. Among antioxidant enzymes, kitchen-waste fertilizer produced the highest catalase activity (159.97 U mg^{-1} protein), whereas Nitrophos produced the highest peroxidase activity (144.77 U mg^{-1} protein). Catalase and peroxidase are components of plant antioxidant systems, but the present experiment did not directly quantify reactive oxygen species or oxidative stress. Consequently, the higher enzyme activities demonstrate differences in antioxidant enzyme activity among treatments but do not, by themselves, establish improved oxidative-stress tolerance.

The relatively higher antioxidant-related responses under organic treatments are consistent with the broader findings of previous studies showing that organic and biological nutrient sources can influence biochemical characteristics of garlic. Alizad et al. (2018) reported differences in physiological characteristics of garlic according to fertilizer type, while Nditasari et al. (2023) examined changes in microbial communities associated with different organic fertilizer applications in garlic. The present results similarly indicate that fertilizer source can affect biochemical characteristics, although the mechanisms responsible for these responses require further investigation.

Relative Water Content and Gas Exchange

Nitrophos recorded the highest relative water content (72.50%), followed by OWDC (71.23%). The highest net photosynthetic rate (15.57 µmol m^{-2} s^{-1}) and transpiration rate (5.67) were also recorded under Nitrophos. These results show that the treatment associated with the highest photosynthetic performance also showed the highest relative water content and transpiration in the present experiment.

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However, the higher relative water content alone cannot explain the differences in photosynthetic performance. OWDC also maintained relatively high relative water content but had a lower photosynthetic rate than Nitrophos. Therefore, several measured factors, including pigment concentration and mineral status, may have contributed to the observed variation, although the present study was not designed to establish their individual contributions.

Comparative Performance and Agricultural Implications

Overall, the results demonstrate contrasting strengths among fertilizer sources. Nitrophos was the strongest treatment for chlorophyll accumulation, photosynthetic performance, soluble protein, and leaf N, P, and K concentrations. Organic treatments, particularly OWDC and kitchen-waste fertilizer, showed higher values for selected biochemical and antioxidant-related parameters, including proline, soluble sugars, ascorbic acid, and catalase activity. Vermicompost generally produced intermediate responses.

These findings suggest that fertilizer selection may depend on the production objective. Chemical fertilization may be useful when the primary objective is to promote rapid physiological performance and mineral accumulation, whereas organic amendments may contribute to selected biochemical and antioxidant characteristics. However, the present study did not evaluate long-term soil health, yield, economic performance, or mixtures of fertilizer sources. Therefore, claims regarding long-term sustainability or superiority of integrated fertilization cannot be directly established from this experiment.

The possibility of integrating chemical, organic, and biological nutrient sources should therefore be considered a **future research direction rather than a conclusion directly tested in the present experiment**. Field studies involving different application rates, fertilizer combinations, larger plant populations, and multiple growth stages are needed to determine whether integrated nutrient management can provide complementary benefits for garlic productivity, plant biochemical characteristics, and soil quality.

CONCLUSION

The results demonstrated that fertilizer source influenced the physiological, biochemical, antioxidant, and mineral characteristics of garlic (*Allium sativum* L.). Nitrophos was the most effective treatment for improving photosynthetic pigments, net photosynthetic rate, total soluble protein, relative water content, and N, P, and K accumulation. In contrast, organic fertilizers, particularly kitchen-waste fertilizer and OWDC, showed greater values for several biochemical and antioxidant-related traits, including total soluble sugars, proline, ascorbic acid, and catalase activity. Vermicompost produced intermediate responses for most of the evaluated parameters.

Overall, the findings indicate that chemical fertilizer provides a stronger short-term advantage for physiological performance and mineral nutrition, whereas organic fertilizers offer advantages for selected biochemical and antioxidant characteristics. Since fertilizer mixtures were not evaluated in this experiment, integrated application of chemical, organic, and biological nutrient sources should be considered as a **potential management strategy for future research**, particularly under field conditions and with different application rates and combinations. Further studies should

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directly test such combinations to determine whether they can improve garlic productivity, soil quality, and long-term nutrient-use sustainability.

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