

## Synergic effect of sulfur and nitrogen on morpho-physiological and biochemical traits of sunflower

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### Abstract

Macronutrients are essential for plant growth and development because they give plants the building blocks, they need to stay healthy and do important biological processes. A completely randomized designed (CRD) pot experiment was done to find out how nitrogen and sulfur affected the morphology and biochemistry of sunflowers. There were three replicates of each treatment: N1 (0), N2 20 kg ha<sup>-1</sup> and N3 40 kg ha<sup>-1</sup> and sulfur S1 (0), S2 40 kg ha<sup>-1</sup> and S3 60 kg ha<sup>-1</sup>. Sunflower growth and physiology were improved by exogenously applied sulfur and nitrogen to the plant. Root and shoot length, fresh and dry weight, number of leaves per plant, leaf area, plant height, chlorophyll *a*, chlorophyll *b*, carotenoids, total soluble protein, and shoot calcium, potassium, and sodium ions contents all went up compared to the control conditions. Furthermore, sulfur and nitrogen have positive impacts on sunflowers' growth, ionic parameters and there was increase for anthocyanin (24.98% and 36.66%), ascorbic acid (43.17% and 97.88%), and flavonoid (34.56% and 112.91%) amounts with the highest levels at 60 kg ha<sup>-1</sup> of S and 40 kg ha<sup>-1</sup> of N, respectively. There was significant increase in photosynthetic pigments on application of Sulphur (Chl *a*: 19.15%, Chl *b*: 52.17%) and Nitrogen (chl *a*: 20.53%, Chl *b*: 57.16%). Thus, applying sulfur and nitrogen externally to the plant is suggested to boost sunflower growth and chlorophyll levels at different nutritional levels.

**Keywords:** nitrogen; photosynthetic pigments; secondary metabolites; sulphur; sunflower

### Introduction

The sunflower (*Helianthus annuus* L.) is one of the most commercial oilseed crops, belonging to the Asteraceae family and the genus *Helianthus*, consist of more than 70 species (Shafique *et al.*, 2022). Sunflower

Received: 26 Dec 2023. Received in revised form: 12 Mar 2024. Accepted: 27 May 2024. Published online: 25 Jun 2024.

From Volume 49, Issue 1, 2021, Notulae Botanicae Horti Agrobotanici Cluj-Napoca journal uses article numbers in place of the traditional method of continuous pagination through the volume. The journal will continue to appear quarterly, as before, with four annual numbers.

is an agricultural commodity that is grown all over the world for its oil production and protein value (Nungula *et al.*, 2023; Özçınar, 2023; Emmanuely *et al.*, 2024). Sunflower seeds are major sources of protein from plants, and their nutritious and beneficial properties have been examined in detail (Seleiman *et al.*, 2020). The sunflower seeds are composed of 18.72% protein, 5.50% moisture, 6.11% carbohydrates, 28.30% crude fiber, 37.47% crude fat, and 3.49% ash. Sunflower cake has a crude fat content 11.0%, crude fiber content 30.18%, a moisture content 5.80%, carbohydrate content 23.75%, and ash content of 5.66% (Kaur *et al.*, 2021). The primary nutritional components of sunflower seeds and oils are responsible for their numerous health benefits (Seleiman *et al.*, 2019). These components have a high levels of monounsaturated and polyunsaturated fats, phytosterols, proteins, tocopherols, vitamin B, antioxidants, antimicrobial, anti-diabetic, anti-inflammatory, and antihypertensive properties, as well as iron, zinc, folate, and copper content (Adeleke *et al.*, 2020).

In Pakistan, sunflower ranks as the third most crucial oilseed crop after cotton, rapeseed, and mustard, but unfortunately, Pakistan produces only 18% of the required edible oil locally among them, sunflower contributes 11% in the local production of oil (Sher *et al.*, 2022). The sunflower yield in Pakistan falls significantly below that of other nations, primarily due to inadequate and improper fertilization practices. While all nutrients play a crucial role, nitrogen (N) stands out as essential because it enhances root and leaf length, extends leaf area duration, promotes photosynthesis, and ultimately boosts seed yield (Mahboob *et al.*, 2023). Numerous factors influence increased yield per hectare, but effective fertilizer management takes precedence in driving sunflower crop growth, development, and achene yield. Insufficient nitrogen availability in sunflowers, especially during their initial growth stages, decreases the number of leaves and hinders leaf expansion (Brock *et al.*, 2023; Rawi *et al.*, 2023). As a result, this leads to a decrease in the leaf area index, overall development, and the capacity to capture sunlight. Similarly, inadequate nitrogen supply also affects the rate of photosynthesis, often causing a reduction in the presence of green pigments and a decline in the efficiency of the Rubisco enzyme (Morales *et al.*, 2018).

The quantity of mineral elements made available to plants may be dramatically altered by using fertilizers. During the initial stage of the growth period, the sunflower's technological cycle undergoes the highest levels of stress (Domaratskyi *et al.*, 2020), it is essential to treat plants with growth hormones in these instances. These growth regulators reduce the adverse effects of stressful circumstances and promote plants to consume a high volume of macro- and micronutrients. Mujeeb-ul-Haq *et al.* (2020) found that sufficient nitrogen is vital for increasing head girth, biological seed yield, 1000-achene weight, and seed yield per head in sunflower plant. Nitrogen production significantly affects food growth because it affects how much leaf area grows and how much photosynthesis happens. According to Zhu *et al.* (2021) there are two sources of nitrogen availability: nitrate ( $\text{NO}_3^-$ ) and ammonium ( $\text{NH}_4^+$ ), both sources impact crop growth, particularly during the early stages of crop development. In the study by Adeleke *et al.* (2020) and Zenda *et al.* (2021) sunflower is believed to be a heavy feeder of nutrients, notably sulfur, which stimulates enzymes that aid in biochemical processes inside plants and raise oil. Although it is believed to be the fourth most vital nutrient, sulfur, for certain plants, is just as essential as phosphorus (P). Oil seed plants need more sulfur than other plants grown on farms. If sunflowers do not have enough sulfur, their growth will slow down, and their grain and oil output will decline (Patel *et al.*, 2019). The way N, P, and S affect each other in sunflowers is beneficial for both its grain production and its oil composition (Mekdad *et al.*, 2021). Leaves become pale, internodes become shorter, and plant height is reduced in sunflowers due to sulfur deficiency. Sulfur (S) does not just stop at its direct benefits; it also works its magic by enhancing the accessibility of other vital nutrients like zinc (Zn), potassium (K), N, and P. This synergistic effect has been well-documented in studies like Bargaz *et al.* (2018), underscoring the role of sulfur in unlocking your soil's potential. Sulfur is the primary element that helps improve sunflower crops' grain quality and how well N and P are applied. Pakistan has highlighted the deficiency of available S in the country's soils (Rana *et al.*, 2022), which has had a detrimental impact on the per-acre yield of sunflower and other oilseed crops.

The synergistic effect of S and N on sunflower cultivation is a dynamic partnership that leads to remarkable outcomes. When applying S and N, they work harmoniously to optimize sunflower growth and yield. N is essential for vegetative growth and overall plant development. At the same time, S plays a pivotal role in amino acid synthesis and chlorophyll formation, which are fundamental processes for photosynthesis and the production of proteins. This collaborative action ensures sunflowers receive a balanced supply of nutrients, resulting in enhanced biomass production, robust stem, and leaf development, improved photosynthetic efficiency, and increased sunflower yields. This synergy underscores the importance of considering S and N in sunflower fertilizer management to maximize crop performance. This study tries to comprehensively investigate the combined impact of sulfur (S) and nitrogen (N) on sunflower's morpho-physiological and biochemical traits. It aims to determine optimal S and N doses for maximizing sunflower growth, yield, and nutrient uptake. The research deepens the understanding of the synergistic relationship between S and N, emphasizing their collaborative impact on sunflower morpho-physiological and biochemical traits. Ultimately, the study proposes practical and sustainable fertilizer management recommendations to optimize sunflower outcomes in Pakistan.

## Materials and Methods

The trial was conducted at University of Agriculture Faisalabad, Postgraduate Agricultural Research Station (Latitude: 31.383456 and Longitude: 72.987833). The sunflower variety FH-516 seeds were obtained from Ayyub Agriculture Research Institute (AARI) Faisalabad, Pakistan, and grown in plastic pots [width (24 cm) and depth (30 cm)] separately filled with 10 kg soil (loamy with moderately alkaline). After thinning, seven seedlings were maintained in each pot with three replicates. Three nitrogen levels (N: 0, 20, and 40 kg/ha) were applied in two steps, half dose of each treatment was mixed in soil of each pot at the time of sowing and then, after three weeks of germination, and sampling was done after four weeks of the last treatment of Sulphur ranging (0, 40, and 60 kg/ha). The sources of nitrogen and sulfur were Urea and calcium sulphate dehydrated respectively. Due to the requirements of the plants, a fully concentrated Hoagland's nutrient solution was applied in every pot. Plants growth attributes include shoot and root length (cm), fresh weight (g), and dry weight (g), number of leaves per plant, leaf area, physiological and biochemical attributes Chlorophyll *a*., Chlorophyll *b*., total chlorophyll contents, chlorophyll *a/b* ratio, carotenoids total soluble proteins, anthocyanin, ascorbic acid, flavonoids, sodium, calcium, and potassium ions were recorded and evaluated.

### *Photosynthetic pigments*

Arnon (1949) protocol was used to determine a photosynthetic pigments by grinding 0.5 g of fresh plant material in 5 mL of 80% acetone, followed by centrifugation and measurement of absorbance at 480 nm, 645 nm, and 663 nm using a spectrophotometer to calculate chlorophylls and carotenoid values formula used by Mounir *et al.* (2021).

$$\text{Chlorophyll } a. (\text{mg /g f. wt.}) = [12.7 (\text{OD } 663) - 2.69 (\text{OD } 645)] \times V/1000 \times W.$$

$$\text{Chlorophyll } b. (\text{mg /g f. wt.}) = [22.9 (\text{OD } 645) - 4.68 (\text{OD } 663)] \times V/1000 \times W.$$

$$\text{Carotenoids (mg /g f. wt.)} = [7.6 (\text{O.D } 480) - 1.69 (\text{O.D } 510)] \times V/1000 \times W$$

Where:

OD=optical density

V= volume of sample (ml)

W= weight of sample (g)

#### *Digestion method*

Shoot and root-dried material (0.1 g) were digested in conc. H<sub>2</sub>SO<sub>4</sub> 2 ml at room temperature overnight, then mixed with H<sub>2</sub>O<sub>2</sub> (4 ml) and heated at 350 °C until colorless by using a procedure given by (Wolf, 1982). The resulting mixture was diluted up to 50 ml, filtered, and analyzed for mineral ions (Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup>) using a flame photometer.

#### *Determination of ascorbic acid, anthocyanin, and flavonoids contents*

Ascorbic acid extraction involved the use of 5% (w/v) metaphosphoric acid, followed by centrifugation at 10,000 g for 15 minutes at 4 °C. Colorimetric assessment was conducted after incubation at 60 °C with sulfuric acid, disodium phosphate, and sodium molybdate, following the method outlined by Khan *et al.* (2008). Absorbance readings were taken, at 660 nm and the ascorbic acid (AsA) content was calculated using a standard curve. Anthocyanin content was calculated using the formula used by Panuccio *et al.* (2016). Fresh leaf sample 20 mg was incubated in 0.5 mL of methanol/HCl (99/1, v/v) at 4 °C for 24 h. After centrifugation at 6,000 × g for 10 min, supernatant optical density was measured at 530 and 657 nm. Lamaison *et al.* (1991) protocol was used for total flavonoids contents estimation. 1 ml portion of the prepared extract, was diluted at a 1/5 ratio with ethanol, then mixed with 4 ml of ethanol. Subsequently, 1 ml of the analyzed solution was combined with 1 ml of AlCl<sub>3</sub> reagent, incubated for 10 minutes, and the absorbance was measured at 430 nm using a UV/Vis spectrophotometer.

#### *Statistical Analysis*

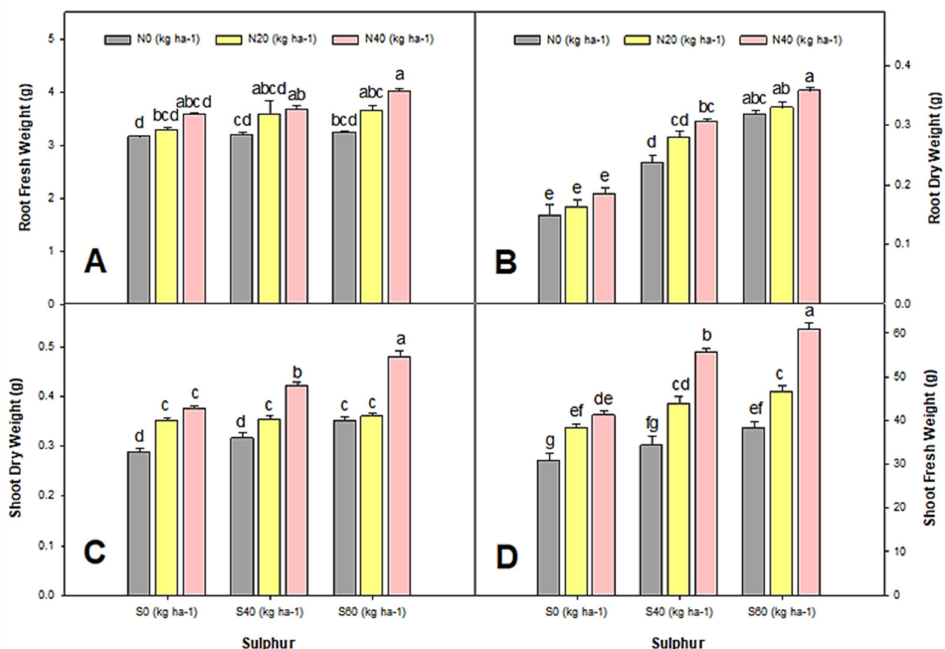
Two-factor factorial ANOVA was performed for each parameter using COSTAT software. Moreover, the Turkey test was used to compare means of simple and interaction effects where applicable at a 5% significance level (Steel *et al.*, 1960).

## **Results**

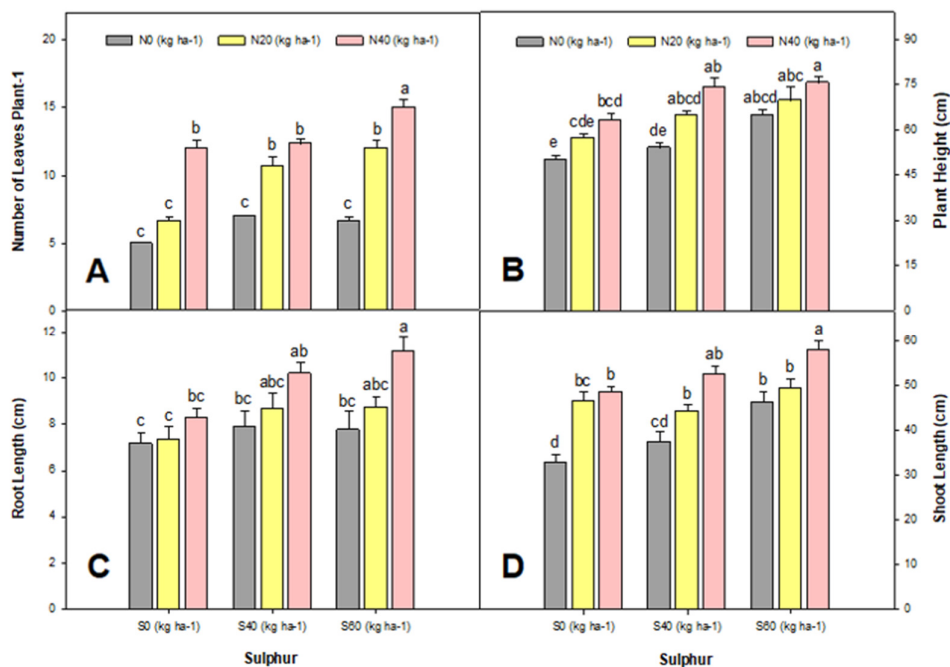
#### *Growth parameters*

When sulfur and nitrogen are combined, they synergistically promote the growth and yield of sunflowers. According to the analysis of variance, the application of exogenous N and S yielded statistically significant results at  $P \leq 0.05$ . Likewise, when sulfur and nitrogen were applied, they showed a significant impact on root and shoot fresh weight, dry weight, and length, and the number of leaves (Figures 1 and 2). However, when considering the interaction between sulfur and nitrogen (S × N), the results were non-significant for plant height with mean values (16.46, ns), root dry weight (0.0003, ns), and shoot length (24.02) (Table 1).

The highest values for growth parameters were recorded in plants treated with 60 kg h<sup>-1</sup> of sulfur and 40 kg ha<sup>-1</sup> of N, while the lowest values for observed growth parameters were noted in pots with no S or N (0 kg ha<sup>-1</sup>) values are given in (Figures 1 and 2). The highest dose 40 kg ha<sup>-1</sup> of N increased the shoot length (36.12%) and root length (29.96%) while sulfur (60 kg ha<sup>-1</sup>) increased root and shoot lengths 19.74% and 21.16% respectively as compared to control values. Similarly, 60 kg h<sup>-1</sup> of S increased the shoot fresh weight (32.38%), shoot dry weight (17.34%) and root fresh weights (8.80%) while 40 kg ha<sup>-1</sup> of N, increased shoot fresh weights, shoot dry weights and root fresh weights 52.41%, 33.50%, and 17.20% respectively as compared to their control levels.



**Figure 1.** Effect of sulphur and nitrogen treatment on root fresh weight (A), root dry weight (B), shoot dry weight (C), and shoot fresh weight (D) of sunflower. The values are mean ( $\pm$ SD) of three replicates.



**Figure 2.** Effect of sulphur and nitrogen treatment on number of leaves (A), plant height (B), root length (C), and shoot length (D) of sunflower. The values are mean ( $\pm$ SD) of three replicates.

Overall, the results showed that a high dose of S and N positively increased sunflower growth data on the means of growth parameters for each treatment. Detailed data on the mean values for each treatment is given in (Table 1).

**Table 1.** Analysis of variance for morphological and biochemical traits of sunflower plants treated with varying doses of sulphur and nitrogen. Ascorbic acid (AA), shoot fresh weight (SFW), anthocyanin (ANT), shoot length (SL), root fresh weight (RFW), flavonoids (FLV.), root length (RL), leaf area (LA), and shoot dry weight (SDW).

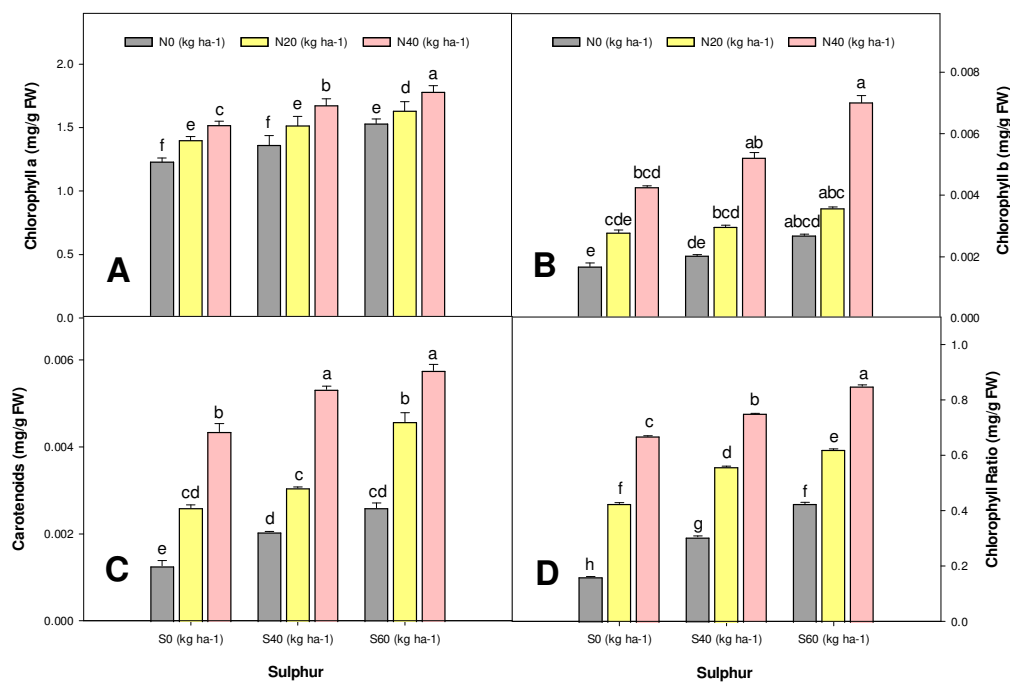
Source	DF	AA	ANT	FLV	RFW	SFW	RDW	SDW	RL	SL	LA
S	2	0.059***	10.18***	0.015***	0.16***	329.26***	0.066***	0.007***	9.195**	174.52***	30119.9***
N	2	0.216***	41.14***	0.097***	0.48***	738.13***	0.005***	0.026***	12.82**	453.25***	10200.7***
S*N	4	0.002**	1.43**	0.001***	0.14***	41.63**	0.0003**	0.001***	9.55**	24.02*	10200.7ns

*Photosynthetic pigments*

The measurements of photosynthetic pigments were taken one week after the last treatment was applied. All results go through statistical analysis, revealing significant outcomes when applying sulfur and nitrogen. For photosynthetic pigments, specifically chlorophyll *b* and carotenoids, the interaction between S and N exhibited significant effects ( $P \leq 0.05$  \*\*\*). When S and N were applied individually, both showed significant effects on chlorophyll *a* given in (Figure 3). However, when applied together, their interaction did not produce a significant effect ( $P \geq 0.05$ ) (Table 2). S plays a crucial role in amino acid synthesis and chlorophyll formation, which are fundamental for photosynthesis and protein production. Compared to the control group, the highest levels of photosynthetic pigments (Chl *a*. :19.15%, and 20.53%, (Chl *b*. 52.17% and 57.16% respectively) were observed in plants treated with the maximum levels of sulfur (60 kg ha<sup>-1</sup>) and nitrogen (40 kg ha<sup>-1</sup>) (Figure 3). Sulphur and nitrogen application on sunflower produced positive effects on photosynthetic pigments well corresponding with the findings of Kalaiyarasan *et al.* (2020) and Najjar *et al.* (2011). Detailed data on the mean values for each treatment given in (Table 2).

**Table 2.** Analysis of variance for morphological and biochemical traits of sunflower plants treated with varying doses of sulphur and nitrogen. Potassium (K<sup>+</sup>), Calcium (Ca<sup>2+</sup>), Sodium (Na<sup>+</sup>), number of leave (NOL), carotenoid (Car), chlorophyll *a* (CHL*a*), chlorophyll *b* (CHL*b*), Chlorophyll ratio (CHLR), and plant height (PH)

Source	DF	K <sup>+</sup>	Ca <sup>2+</sup>	Na <sup>+</sup>	CHL <i>a</i>	CHL <i>b</i>	CHLR	CAR	PH	NOL
S	2	41.92***	63.81***	76.77***	0.158***	5.38***	0.102***	5.55***	365.36***	25.59***
N	2	140.03***	61.81***	96.77***	0.180***	2.69***	0.474***	2.269***	445.21***	106.81***
S*N	4	3.20 ns	0.25 ns	3.55 ns	0.001**	8.74***	0.001***	2.75**	16.46*	4.53***

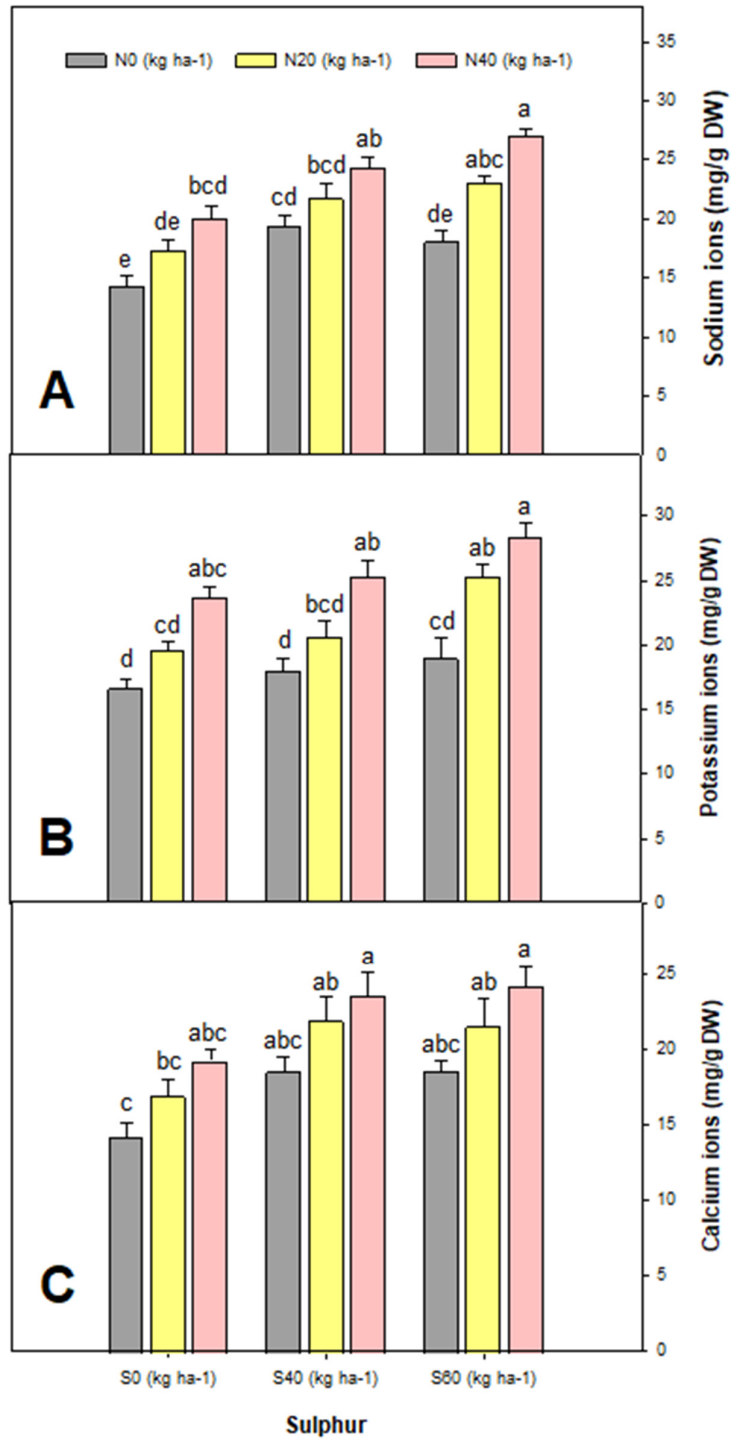


**Figure 3.** Effect of sulphur and nitrogen treatment on chlorophyll *a* (A), chlorophyll *b* (B), carotenoids (C), and chlorophyll ratio (D) of sunflower. The Values are mean ( $\pm$ SD) of three replicates.

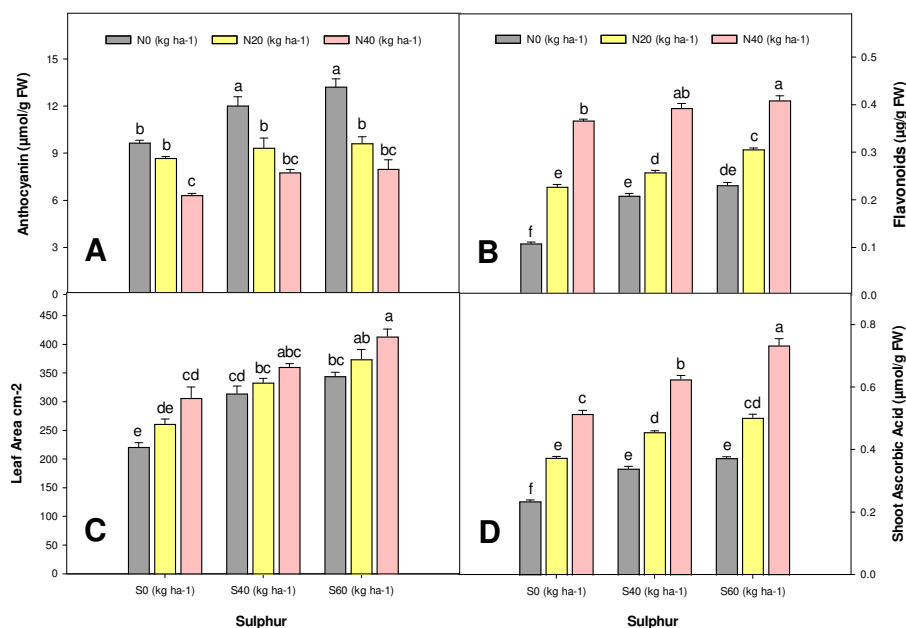
*Ionic content and biochemical traits*

All results of ionic content were subjected to statistical analysis. Mean square values indicated a significant effect for S and N when considered individually (Table 2 and Figure 4). Additionally, a significant effect ( $P \leq 0.05$ ) was observed for potassium ions ( $K^+$ ), and a similar pattern was noticed for calcium ( $Ca^{2+}$ ) and sodium ( $Na^+$ ) when applied individually. However, when S and N were applied together (interaction among  $S \times N$ ), the results were non-significant ( $P \geq 0.05$ ) for  $K^+$ ,  $Ca^{2+}$ , and  $Na^+$  ions, with mean square values of 3.20 (ns), 0.25 (ns), and 3.55 (ns), respectively (Table 2).

Notably, a significant increase was observed between the mean square values for flavonoids (N: 112.91%, S: 34.56%) and ascorbic acid (N: 97.88%, S: 43.17%) as compared to their control groups (Figure 5). For anthocyanins, maximum levels of S (24.98%) and N (36.66%) yielded significant results. However, when examined in combination (interaction among  $S \times N$ ), the result was non-significant with a mean square value of 1.43 (ns). The data about the means of parameters of each treatment are shown in (Table 1 and Figure 5).



**Figure 4.** Effect of sulphur and nitrogen treatment on number of sodium ions (A), potassium ions (B), and calcium ions (C) of sunflower  
The Values are mean ( $\pm$ SD) of three replicates.



**Figure 5.** Effect of sulphur and nitrogen treatment on anthocyanin (A), flavonoids (B), leaf area (C), and ascorbic acid (D) of sunflower

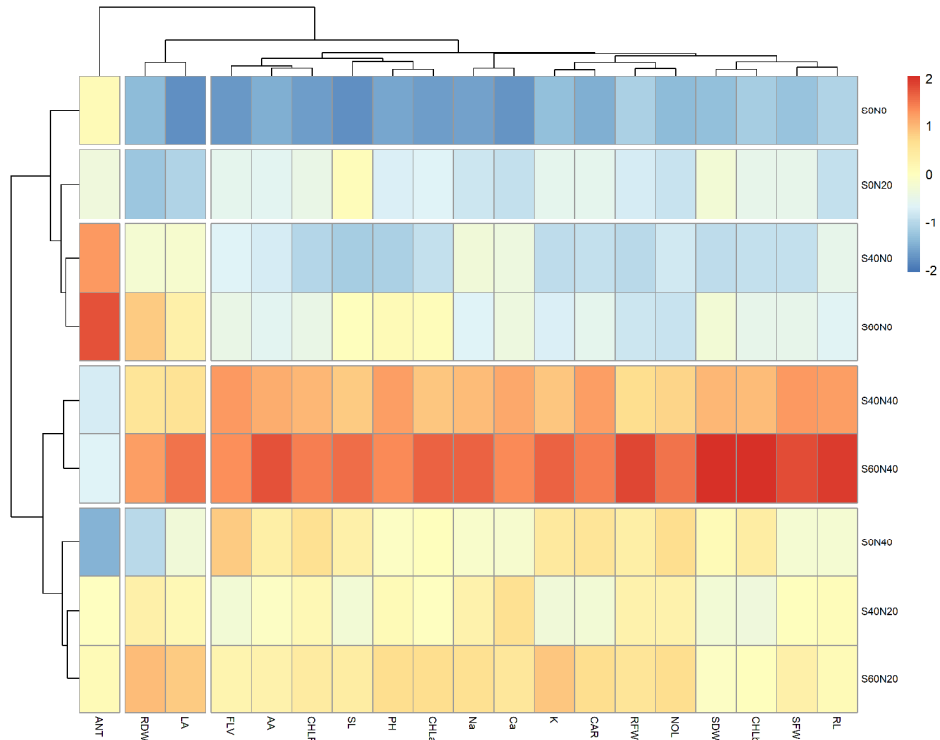
The values are mean ( $\pm$ SD) of three replicates.

### Heatmap

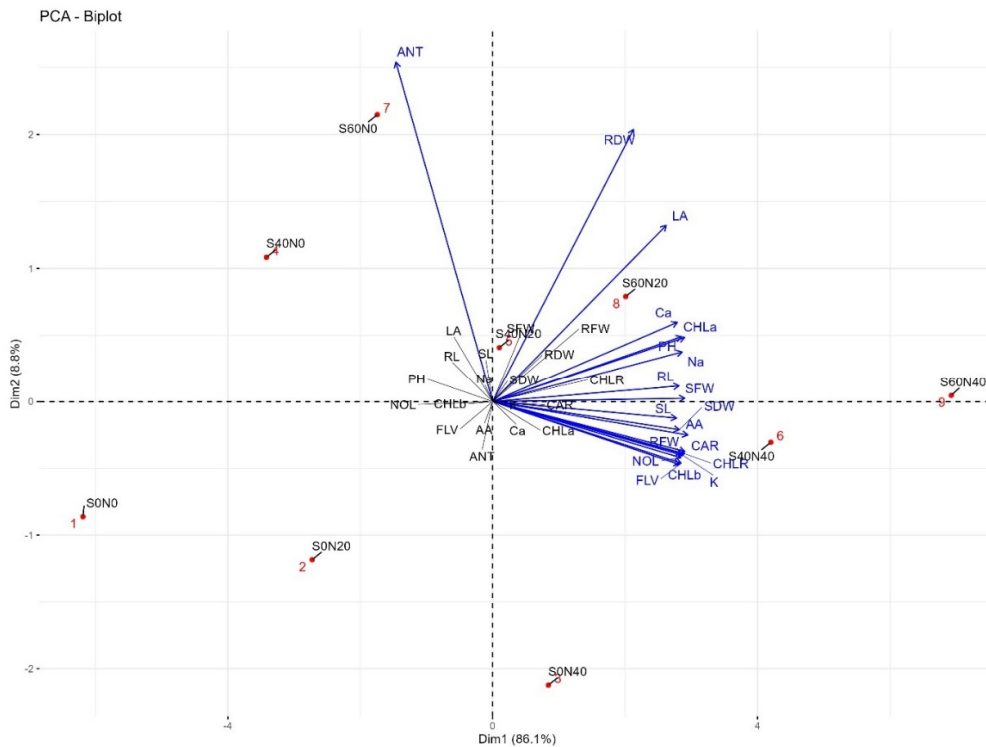
The heatmap illustrates associations between morpho-physiological and biochemical parameters at three nitrogen levels (0N, 20N, 40N) and sulfur levels (S0, S40, S60) (Figure 6). The major cluster has three sub-clusters. The first sub-cluster shows a strong positive association of anthocyanin with N0S40 and N0S60. The second sub-cluster demonstrates a strong positive association between root dry weight and leaf area at N40S60, with a strong negative grouping at N0S0. The third sub-cluster exhibits a strong negative association of biochemical traits (flavonoids, amino acids, chlorophyll ratio) with shoot length, plant height, chlorophyll a, plant Na<sup>+</sup>, and Ca<sup>2+</sup> at N0S0. Furthermore, biochemical traits (flavonoids, amino acids, chlorophyll ratio, chlorophyll a and b, Na<sup>+</sup>, Ca<sup>2+</sup>, K<sup>+</sup> ions, carotenoids) show a strong positive relationship with shoot length, plant height, shoot fresh and dry weight, root length, number of leaves per plant, and root fresh weight at N40S60.

### PCA

Principal component analysis between morpho-physiological and biochemical traits revealed three isolated clusters (Figure 7). The first cluster involved morphological traits, such as leaf area, showing a weak association with root dry weight, while biochemical traits, including chlorophyll a, Ca<sup>2+</sup>, and Na<sup>+</sup>, were strongly associated with plant height, root length, and shoot fresh weight at S60N20. The second cluster showed a weak link between anthocyanin and S60N0, as well as S40N20. The third cluster exhibited strong associations between biochemical traits, such as amino acids, carotenoids, chlorophyll ratio, chlorophyll b, flavonoids, and K<sup>+</sup>, and variables like shoot length, shoot dry weight, root fresh weight, and the number of leaves per plant at S40N40.



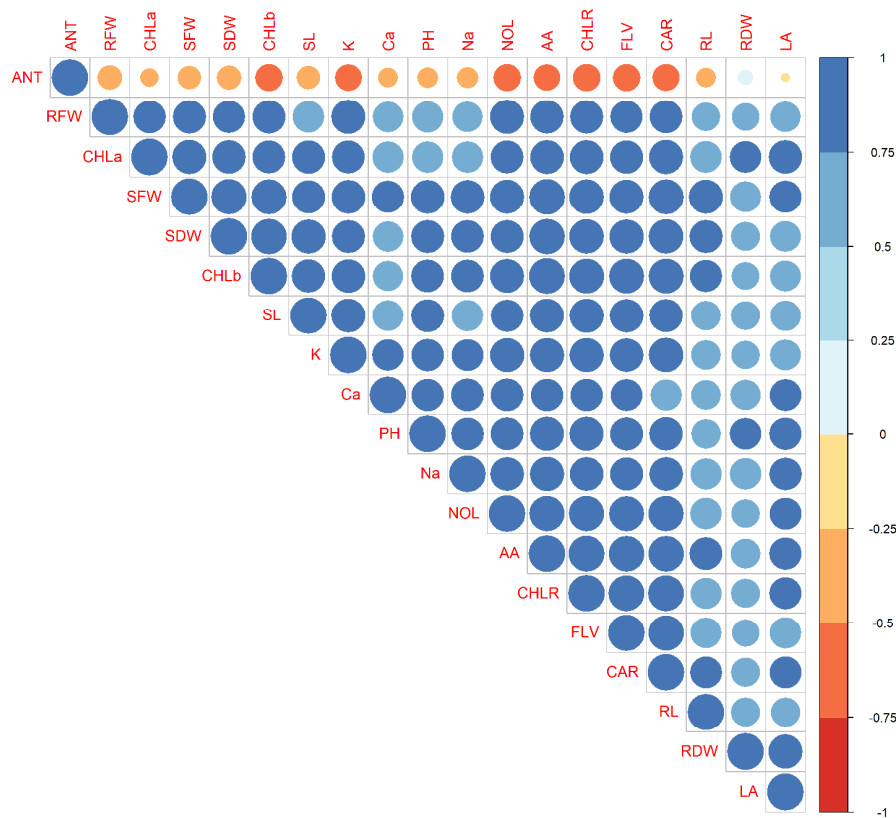
**Figure 6.** The heatmap illustrates associations between morpho-physiological and biochemical parameters of sunflower at applied nitrogen and Sulphur levels.



**Figure 7.** Principal component analysis (PCA), between morpho-physiological and biochemical traits of sunflower

*Correlation*

The correlation between morpho-physiological and biochemical traits is presented in (Figure 8). Anthocyanin exhibits a strong negative correlation with chlorophyll *b*, K<sup>+</sup> ions, the number of leaves per plant, amino acids, chlorophyll ratio, flavonoids, carotenoids, and root length. The number of leaves per plant shows a positive correlation with root fresh weight, chlorophyll *a*, shoot fresh and dry weight, chlorophyll *b*, shoot length, K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup> ions, and plant height. Biochemical traits, such as amino acids, show a negative correlation with anthocyanin and are positively correlated with root fresh weight, chlorophyll *a*, shoot fresh and dry weight, chlorophyll *b*, K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup> ions, plant height, and the number of leaves per plant. Chlorophyll ratio shows a negative correlation with anthocyanin and is positively correlated with root fresh weight, chlorophyll *a*, shoot fresh and dry weight, chlorophyll *b*, shoot length, K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup> ions, plant height, the number of leaves per plant, and amino acids. Flavonoids show a negative correlation with anthocyanin and are positively correlated with root fresh weight, chlorophyll *a*, shoot fresh and dry weight, chlorophyll *b*, K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup> ions, plant height, the number of leaves per plant, amino acids, and chlorophyll ratio. Carotenoids show a negative correlation with anthocyanin and are negatively correlated with root fresh weight, chlorophyll *a*, shoot fresh and dry weight, chlorophyll *b*, K<sup>+</sup>, Ca<sup>2+</sup>, Na<sup>+</sup> ions, plant height, the number of leaves per plant, amino acids, chlorophyll ratio, and flavonoids."



**Figure 8.** The correlation between morpho-physiological and biochemical traits of sunflower

## Discussion

Adding S and N to the growing medium significantly improved sunflower growth, yield, and crop traits. According to Leghari *et al.* (2016), the application of nitrogen affects the dry matter and the distribution of nitrogen throughout the crop plant, which in turn affects the growth, development, and other vital activities. The 40 kg N ha<sup>-1</sup> and 60 kg S ha<sup>-1</sup> were the levels of nitrogen and sulfur that performed the best in terms of growth characteristics in the current study. These levels resulted in a significant increase in plant height (76 cm), as well as root and shoot fresh and dry weights, which correlated to the findings of Ahmad *et al.* (2018) and Jarecki (2022). Sarkar *et al.* (2009) and Sarkar *et al.* (2009) suggest that S and N work together to boost sunflower growth and yield by encouraging vegetative growth and plant development.

Photosynthesis and protein production depend on S for amino acid synthesis and chlorophyll formation (Ramyar *et al.*, 2023; Shah *et al.*, 2022). The presence of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids) was shown to have a substantial impact. This is since nitrogen can induce the growth of more leaves and increase the total plant height. Since additional leaves generate more chlorophyll, the total chlorophyll content of the plant may result in an increase. This has the potential to result in increased agricultural yields as well as an increase in the production of outstanding biomass. According to Vwioko *et al.* (2018) adding sulfur and nitrogen to sunflowers indirectly helps them make ascorbic acid (or vitamin C) because they both boost the production of different nutrients, which in turn speeds up biological processes. Ascorbic acid production is improved in healthy plants because they are better at controlling their metabolism. Tuladhar *et al.* (2021) say that sulfur is essential for making flavonoids and for plants' defence systems.

Providing the right amount of nutrients helps plants grow in the best way, which may improve the production of flavonoids. According to Jamal *et al.* (2010), molecular traits like flavonoids (0.001\*\*\*), anthocyanin, and ascorbic acid (0.002) had significant effects on the consumption of both nutrients in sunflowers, either individually or if combined. Another experimental result of Haq *et al.* (2023) and Mussarat *et al.* (2021) revealed that the best outcomes came from using 40 kg N ha<sup>-1</sup> and 60 kg S ha<sup>-1</sup> together. The capacity of sulphur and nitrogen to boost sunflowers' development and output indicated the synergic effect of these two elements. Crucial processes like photosynthesis and protein building depend on sulfur's role in making amino acids and chlorophyll (Narayan *et al.*, 2022). Ascorbic acid, flavonoids, anthocyanin, and photosynthetic pigments like chlorophyll *a*, *b*, and carotenoids were all affected by this interaction. Not only were sunflowers affected by the strong interaction between sulfur and nitrogen, but so were rapeseed, mustard, and safflower crops. The intake of these two nutrients in plants is closely linked because they are necessary for protein production (Ahmad *et al.*, 2018). Plants grow faster during the vegetative and reproductive stages when they get enough nitrogen. This results in higher yields in the final stages of growth. The relationship between sulfur and nitrogen is even more critical in this situation, especially regarding oil yield and overall production. It has been seen that sulfur and nitrogen work well together in rapeseed, mustard, and sunflower plants. Some crops don't get enough sulfur, which lowers their growth and quality and makes nitrogen, phosphorus, and potassium nutrients less effective. This is especially important because soybean crops need more sulfur than other crops.

Our results are comparable to the findings of previous research. For instance, Sincik *et al.* (2013) found that when three different nitrogen levels (100, 150, and 200 kg ha<sup>-1</sup>) were given to sunflowers, the ones with the most seeds, oil, and protein were the ones with 200 kg N ha<sup>-1</sup>. Maragatham *et al.* (2000) found that 120-60-50 kg NPS ha<sup>-1</sup> gave the most sunflower seeds. According to Ahmad *et al.* (2018), sunflower plants grew the tallest, had the widest heads, and weighed the most seeds when they were given 80 kg of nitrogen per acre.

Additionally, Ozer *et al.* (2004) found that rising sulfur levels were linked to sunflower plants growing taller. Hassan *et al.* (2007) found that head sizes got bigger as sulfur treatment went up. Furthermore Hussain *et al.* (2011) found that sulfur application led to the tallest plants and the most dry matter in sunflowers at

harvest. Additionally, Wani *et al.* (2001), Pati *et al.* (2011) and Rana *et al.* (2015) all found that adding sulfur to sunflowers increased the number of achene produced. Rasool *et al.* (2013) and Ravikumar *et al.* (2016) found that adding sulfur to sunflowers increased the weight of 1000 seeds and the amount of oil they contained (%). Researchers have investigated how sulfur can help plants grow and produce more sugar.

### **Conclusions**

According to the results, managing sulfur, nitrogen, and the interaction between sulfur and nitrogen is very important for growing sunflowers successfully. In terms of necessary nutrients for sunflower growth, nitrogen and sulfur are the most important ones. Sulfur deficiency must be fixed to improve the quality and yield of soybean crops, as shown by the higher demand for sulfur. Almost 60% of problems with sunflower farming that stop the plants from growing are caused by not having enough nutrients. To get the best sunflower production, agricultural experts need to know how nutrients combine with one another and how to change fertilizer recommendations.

### **Authors' Contributions**

Conceptualization: AM and MSF. Writing original draft: KJ and MSU. Reviewing and editing: AM, MSF, NA, AA, MA Funding acquisition MSF. All authors read and approved the final manuscript.

### **Ethical approval** (for researches involving animals or humans)

Not applicable.

### **Acknowledgements**

Researchers Supporting Project number (RSPD2024R751), King Saud University, Riyadh, Saudi Arabia is acknowledged.

## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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