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# Investigating the Synergistic Effects of Using Nitrogen and Sulfur Fertilizers on the Growth of Safflower (*Carthamus tinctorius* L.)

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**Abstract:** Nutrient imbalance is one of the prevalent problems in the semi-arid region. The study aimed to investigate the effects of sole and simultaneous application of nitrogen and sulfur on safflower yield in a semi-arid region. A field trial was designed to explore the combined use of sulfur ($S_0$: control, $S_1$ and $S_2$: 25 and 50 kg ha$^{-1}$ S via sulfur phosphate composites, $S_3$ and $S_4$=: 25 and 50 kg ha$^{-1}$ of S via elemental sulfur, $S_5$ and $S_6$: 25 and 50 kg ha$^{-1}$ of S via zinc sulfate) and nitrogen (0, 40 and 80 kg ha$^{-1}$) on the growth and seed production of safflower (*Carthamus tinctorius* L.) in a west of Iran with mesic active calcixerepts soil that had medium to low fertility. The utilization of large amounts of nitrogen greatly increased the height of the first pod from the ground surface ($P \leq 0.05$), and the highest amount was recorded in $S_6$+ $N_{80}$ conditions (65%). Leaf chlorophyll content was significantly affected by nitrogen consumption ($P \leq 0.01$) and the highest chlorophyll was recorded in $S_4$, $S_5$, and $S_6$ treatments having high levels of nitrogen. A similar trend was observed for the canopy width and the application of nitrogen, zinc sulfate, and elemental sulfur could increase this component by 33%. The highest number of heads was achieved with the use of $S_5$ + $N_{80}$ and $S_2$ + $N_{80}$, which was 15% more compared to the control. The application $S_2$ + $N_{80}$ increased seed number in capitulum by 25% over the control. The application of S along with high levels of nitrogen improved the seed yield by 45% (1334 ± 78.35 kg ha$^{-1}$). However, only the application of sulfur could improve the harvest index ($P \leq 0.05$). The results indicated that applying moderate to high amounts of zinc sulfate and elemental sulfur, in combination with N80, enhanced safflower growth characteristics and improved seed yield components, likely due to synergistic interactions between the elements. Altogether the use of zinc sulfate or elemental sulfur as a logical and effective management solution can increase the efficiency of nitrogen fertilizer and improve nutritional balance.

**Key words:** Antagonistic, canopy growth, elemental sulfur, integrated application, soil reclamation, zinc sulfate.



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The safflower is a native plant of semi-arid regions and is an annual herbaceous plant of the Asteraceae family. Safflower (*Carthamus tinctorius* L.) is an annual oilseed crop, however, it is also used for other purposes such as extracting dye from petals, using it as forage in the vegetative stage, and feeding poultry (Emongor and Emongor, 2023). This plant can produce significant green biomass during the vegetative growth phase very fast. Although it does not produce a very high forage yield, its shoot has high nutritional content and due to its unique characteristics, it has adapted well to semi-arid areas. This plant appears to have originated westward toward the Mediterranean Sea, a region characterized by a semi-arid climate with dry, rainless summers and frequent challenges related to nutrient deficiency and water scarcity (El-Lattief, 2012). The root system of the safflower plant can penetrate the soil to a depth of up to 2 meters, which partially explains its ability to resist drought conditions. Although there is some information about how roots grow in the soil, there is still no comprehensive science about how safflower can absorb elements from the rhizosphere. However, not enough studies have been done on the nutritional aspects of safflower. The lack of sufficient information on how to manage plant nutrition and fertilizer management is another limiting factor for the lack of successful development and cultivation of this plant in the world. These factors have caused the safflower plant to be considered an underutilized and neglected crop, although it is the oldest cultivated annual oilseed crop (Pearl and Burke, 2014; Emongor and Emongor, 2023).

However, soils in semi-arid regions face numerous challenges, including high pH levels, irregular precipitation patterns, low organic matter content, overgrazing, minimal return of plant residues to the soil, and poor crop management practices, including unprincipled crop rotation (Quiroga *et al.,* 1998). The requirement to increase food production in these areas will need the planning to change some agricultural practices. In these regions, high levels of calcium and pH can cause nutrient deficiencies (Ayangbenro and Babalola, 2021). In such a situation, reclamation is a necessary management to improve the production and increase the productivity of fertilizers (Hemmaty *et al.,* 2012). Although the sulfur oxidation process can occur naturally in the soil, this natural process is slow and requires the presence of some bacteria, including *Thiobacillus* spp. (Gupta and Germida, 2021). However, it has been reported that under low soil moisture content, the total biomass of *Thiobacillus* spp. is significantly reduced due to nutrient limitation (Naylor and Coleman-Derr, 2018). Therefore, the use of chemical fertilizers containing sulfur should be considered as a rational and reclamation option in dry areas.

The greatest advantage of sulfur application in semi-arid areas is its ability to lower soil pH. This changes the rhizosphere environment and enhances the availability of essential nutrients, contributing to optimal crop growth (Mustafa *et al.*, 2022; Derafshi *et al.*, 2023). Among the nutrients required for the plant, nitrogen has a critical role in most physiological processes related to growth and development (Anas *et al.*, 2020). Most plant nitrogen is invested in the structure of Rubisco, and without a doubt, changes in nitrogen availability can affect the rate of photosynthesis. Nitrogen, the most essential nutrient in plants, is involved in the processes of protein synthesis, and due to the structural and functional role of proteins, its deficiency can severely affect growth (Tiong *et al.*, 2021). Meanwhile, in the soils of semi-arid regions, the availability of this element can be influenced by the presence of other elements or the indirect effects of elements such as sulfur, which has not been well explored so far (Mustafa *et al.*, 2022). Nitrogen, as a key component, plays a crucial role in the biosynthesis of chlorophyll and other essential compounds, such as plant hormones. However, poor soil conditions in semi-arid regions often hinder plants' ability to effectively absorb and utilize applied nitrogen (Ayangbenro and Babalola, 2021). The interaction between nitrogen and sulfur and its impact on the growth of safflower plants in semi-arid regions remains unclear. Understanding this interaction is vital for improving nitrogen use efficiency by enhancing soil conditions through sulfur application. Given the benefits of soil reclamation with sulfur, this study aims to evaluate the effects of sulfur and nitrogen fertilizer applications on safflower plant growth in western Iran.

## Materials and Methods

### *Climatic characteristics of the studied site*

The present experiment was conducted in the research farm located in the semi-mountainous region of Baneh in the west of Iran during 2021 growth season. The geographical coordinates of the area were 35°59′ N and 45°53′ E, 1615 m height above msl. This semi-mountainous region has a semi-dry mountainous climate and most of the rain falls during November to April. The total rainfall during the investigated cropping season was 489 mm. The coldest month of the year at the meteorological station was in February with an average temperature of 2.5°C and the hottest month of the year was August with an average temperature of 24.6°C. The average annual evaporation and transpiration according to the Blaney- Criddle method was more than 1,350 mm. The average number of frosty days during the year was 134 days and the highest wind speed was 55 to 68 km $hr^{-1}$ with southwest and west direction. Average annual sunny hours were estimated to be 2667 hours (Hanafi and Hatami, 2013).

*Soil properties of the field*

The soil of the site was fine mixed, mesic active calcixerepts. To comply with the principle of uniformity of the test material, all preparative operations were carried out on the same test site. A section of the field, measuring 16 × 30 $m^2$, was selected for sampling. Composite soil samples were collected from a depth of 0-30 cm and dried. Various physical and chemical characteristics were assessed, including phosphorus, measured using Olsen's method (Jones, 2001); absorbable potassium, determined by extraction with 1 N ammonium acetate (Jones, 2001); soil texture, analyzed using the two-time hydrometer method (Gee and Bauder, 1986); and organic carbon, assessed using the oxidizing method (Nelson and Sommers, 1996). Additionally, pH was measured in a 1:1 water-to-soil suspension (McLean, 1982), electrical conductivity (EC) was determined in a 1:1 water-to-soil solution (Jones, 2001), calcium carbonate equivalent was evaluated through neutralization with acid and titration with soda (Richards, 1954), and soil sulfate was measured using the monocalcium phosphate method (Jones, 2001). The soil of the field was sandy clay loam, and its physical and chemical characteristics were pH: 7.68, organic carbon: 0.51%, total nitrogen: 0.26%, $CaCO_3$: 17%, EC: 2.15 dS $m^{-1}$, $SO_4^{2-}$: 3.42 mg $kg^{-1}$, P: 16.28 mg $kg^{-1}$, K: 625 mg $kg^{-1}$.

*Fertilizers application method and experimental treatments*

This research was conducted as a factorial experiment, in the form of a randomized block design and three replications. The sulfur factor, the first variable, included seven levels: S0 (control), S1, and S2 representing 25 and 50 kg $kg^{-1}$ of sulfur applied via sulfur phosphate composites; S3 and S4 representing 25 and 50 kg $kg^{-1}$ of sulfur applied via elemental sulfur; and S5 and S6 representing 25 and 50 kg $kg^{-1}$ of sulfur applied via zinc sulfate. Sulfur fertilizer was used during weathering and stacking in the middle of the stack and the root development area 20 days before planting. Land preparation operations, including reversible moldboard plowing and disc plowing, were carried out to ensure completely fine surface soil on February 15, 2021. Then the trial field was divided into experimental plots and the size of each plot was 4 × 4 m. Sulfur fertilizers were used during the formation of ridges and furrows, and they were used in the middle of the ridges and the around root development area 20 days before planting. The distance between the ridges was 50 cm and the ridge height was around 30 cm. Seed planting was done by hand on the ridges at a depth of 5 cm. The second factor included the consumption of different levels of nitrogen (0, 40, and 80 kg $ha^{-1}$) through the urea fertilizer. Nitrogen was used in three installments in pre-planting, stem elongation, and the capitulum emergence stages. The field was irrigated immediately after planting. During the growing season, drip irrigation was carried out through the tapes located on the side of the ridges according to water needs and climatic conditions. All agricultural operations in the growing stage, including weed control, pest control, thinning, hand weeding, and breaking the soil crusts, were done regularly and uniformly for all plots.

*Measurement of investigated traits*

Non-destructive measurement of leaf chlorophyll was performed with the SPAD-502 meter on upper-developed leaves during the capitulum growth stage. The width of the canopy was measured using a ruler from the tip of the left horizontal stem to the tip of the right stem as the diameter of the canopy. Assuming the shape of the canopy to be elliptical from above, the diameter of the ellipse was considered as the width of the canopy. The height of the plant was determined as the height of the plant from the ground to the tip of the highest capitulum at the maturity stage. Plants reached to maturity stage approximately

*Table 1. The mean comparison of growth characteristics of safflower under application of nitrogen and sulfur in semi-arid region*

| | | PH | FCH | SD | GC | BYM | SS |
|---|---|---|---|---|---|---|---|
| $N_0$ | $S_0$ | 47.3 ± 2.00 | 33.7 ± 1.61 | 7.00 ± 1.000 | 66.0 ± 0.87 | 5,921.4 ± 391.81 | 5.3 ± 0.29 |
| | $S_1$ | 52.0 ± 1.73 | 36.0 ± 2.18 | 7.67 ± 0.289 | 68.3 ± 0.29 | 6,917.2 ± 59.39 | 6.0 ± 1.00 |
| | $S_2$ | 53.3 ± 3.21 | 34.0 ± 1.00 | 7.00 ± 0.500 | 70.3 ± 2.08 | 7,506.9 ± 502.43 | 6.0 ± 0.87 |
| | $S_3$ | 53.0 ± 2.65 | 38.3 ± 1.61 | 6.33 ± 0.289 | 70.3 ± 0.76 | 6,416.3 ± 362.03 | 5.7 ± 0.76 |
| | $S_4$ | 62.7 ± 2.89 | 43.3 ± 5.27 | 8.00 ± 0.866 | 77.0 ± 1.80 | 7,214.6 ± 405.41 | 4.7 ± 0.29 |
| | $S_5$ | 63.3 ± 5.13 | 41.3 ± 3.97 | 7.00 ± 0.866 | 78.0 ± 3.77 | 6,391.3 ± 181.54 | 5.0 ± 0.87 |
| | $S_6$ | 66.7 ± 3.21 | 46.0 ± 5.51 | 8.33 ± 0.289 | 75.7 ± 2.75 | 6,831.0 ± 70.24 | 4.0 ± 0.00 |
| $N_{40}$ | $S_0$ | 54.7 ± 3.61 | 35.7 ± 1.44 | 7.33 ± 0.289 | 69.7 ± 0.29 | 6,380.7 ± 247.29 | 4.3 ± 0.29 |
| | $S_1$ | 56.0 ± 3.00 | 36.0 ± 2.25 | 8.67 ± 0.289 | 76.0 ± 0.87 | 6,532.5 ± 305.29 | 4.7 ± 0.58 |
| | $S_2$ | 59.3 ± 2.65 | 38.7 ± 2.75 | 8.33 ± 0.764 | 78.3 ± 1.44 | 7,191.3 ± 305.68 | 4.7 ± 0.29 |
| | $S_3$ | 65.7 ± 9.87 | 44.3 ± 3.62 | 7.33 ± 1.155 | 76.3 ± 2.75 | 7,567.9 ± 130.78 | 5.7 ± 0.76 |
| | $S_4$ | 66.3 ± 2.52 | 40.3 ± 4.77 | 7.33 ± 0.577 | 78.3 ± 1.44 | 7,106.3 ± 345.65 | 5.3 ± 0.29 |
| | $S_5$ | 65.7 ± 6.24 | 41.0 ± 4.37 | 7.67 ± 0.289 | 79.0 ± 1.80 | 6,756.5 ± 231.99 | 5.7 ± 0.29 |
| | $S_6$ | 70.3 ± 5.51 | 42.3 ± 4.54 | 7.00 ± 0.866 | 80.0 ± 2.50 | 6,720.6 ± 421.25 | 4.7 ± 0.29 |
| $N_{80}$ | $S_0$ | 59.0 ± 1.15 | 41.0 ± 1.00 | 8.33 ± 1.155 | 74.3 ± 0.58 | 7,173.1 ± 455.00 | 5.0 ± 0.50 |
| | $S_1$ | 60.3 ± 1.53 | 41.0 ± 0.76 | 8.67 ± 1.041 | 79.7 ± 2.52 | 7,647.7 ± 932.46 | 5.0 ± 0.87 |
| | $S_2$ | 66.3 ± 2.52 | 38.3 ± 2.78 | 8.33 ± 0.289 | 78.3 ± 1.44 | 8,757.7 ± 773.40 | 5.7 ± 1.04 |
| | $S_3$ | 66.7 ± 7.02 | 42.3 ± 1.44 | 6.67 ± 0.289 | 78.3 ± 1.44 | 7,979.5 ± 554.92 | 5.3 ± 0.29 |
| | $S_4$ | 71.3 ± 12.22 | 48.3 ± 3.06 | 7.33 ± 0.289 | 84.7 ± 0.29 | 7,744.5 ± 332.39 | 5.7 ± 0.29 |
| | $S_5$ | 76.7 ± 1.15 | 45.3 ± 2.57 | 8.00 ± 0.500 | 87.0 ± 1.32 | 7,355.2 ± 118.15 | 4.3 ± 0.29 |
| | $S_6$ | 79.7 ± 8.62 | 53.0 ± 1.89 | 8.67 ± 0.289 | 84.3 ± 2.52 | 7,765.6 ± 107.59 | 4.0 ± 0.12 |
| LSD | | 7.92 | 9.61 | 2.11 | 6.17 | 1,315.7 | 3.78 |
| Significance level | | | | | | | |
| N | | 0.001 | 0.011 | 0.045 | 0.001 | 0.000 | 0.716 |
| S | | 0.001 | 0.000 | 0.279 | 0.001 | 0.037 | 0.203 |
| N×S | | 0.058 | 0.609 | 0.805 | 0.839 | 0.931 | 0.610 |

Note: $N_0$: no nitrogen application, $N_{40}$: application of 40 kg ha$^{-1}$ nitrogen, $N_{80}$: kg ha$^{-1}$ nitrogen, $S_0$: control, $S_1$ and $S_2$: 25 and 50 kg ha$^{-1}$ S via sulfur phosphate composites, $S_3$ and $S_4$- 25 and 50 kg ha$^{-1}$ of S via elemental sulfur, $S_5$ and $S_6$: 25 and 50 kg ha$^{-1}$ of S via zinc sulfate. The numbers represent the mean ± standard deviation Plant height: PH (cm), FCH: The height of the first capitulum from the ground level (cm), PSY: plant seed yield, CW: canopy width, SBN: number of the secondary branch, GC: ground cover percentage, BYM: biological yield, SD: stem diameter, SS: percentage of shriveled seeds. At the significance level P value are presented the numbers smaller than one and five are significant at the statistical level of one and five percent, respectively. Numbers greater than 5 indicate that the mentioned treatment is statistically insignificant. If the difference between two means in each column is more than the LSD, then those means differ significantly.

30 days after flowering, when most leaves have turned brown. The number of capitula (heads) per plant, capitulum diameter, and the number of branches were counted for ten randomly selected plants before harvesting. After harvesting the plants from 2 m$^2$ the number of seeds in the capitulum and seed yield were counted. Plants were placed in a drying cabinet at 70°C for 24 hrs, and the total dry matter (biological yield) was measured through a digital scale. The thousand seed weight was obtained by counting the seeds with a seed counter and weighing them. The harvest index was obtained through the ratio of grain yield to total dry matter.

### *Statistical analysis of data*

Statistical analysis of data was done using MSTATC and SPSS software. First, the data distribution was tested for normality, and if necessary, appropriate transformation was used for non-normal data. The analysis of variance was performed based on the randomized complete block design in factorial layout, whereas fertilizers' treatments (various levels of sulfur and nitrogen) as well as replication were assumed as fixed factors. The comparison of the means was done with the least significant difference test at the 5% probability level and the figures were drawn using SPSS software.

## Results and Discussion

The results of the analysis of variance showed that plant height was affected by nitrogen consumption ($p<0.01$), consumption of 40 kg and 80 kg ha$^{-1}$ of nitrogen increased plant height by 13% and 28%, respectively. On the other hand, the consumption of sulfur, especially zinc sulfate, could increase the height ($p<0.01$). The tallest plants were obtained under the conditions of application of $N_{80}$ along with the use of $S_4$, $S_5$, and $S_6$, which was about 30% higher than the control (Table 1).

The investigation of the height of the first capitulum from the ground level (FCH) showed that nitrogen effects on this trait was significant ($p<0.01$). However, the interaction effects of these two factors not statistically insignificant ($p>0.05$). Means comparison showed that the highest FCH was recorded for plants grown by $N_0S_3$ and those grown by of 50 kg ha$^{-1}$ of zinc sulfate along with 80 kg of nitrogen (Table 1). Nitrogen consumption affected the stem diameter ($p<0.05$). The application of large amounts of nitrogen increased the stem diameter by about 8%. Evaluation of number of secondary branches (SBN) showed the interaction effect of nitrogen × sulfur was significant ($p<0.05$) and the highest SBN was obtained under the conditions of application of $S_3N_{80}$, $S_5N_{80}$, $S_6N_0$, $S_6N_{40}$, and $S_6N_{80}$ (Fig. 1). The results indicated that the application of zinc sulfate significantly improved this trait. Interestingly, the application of 50 kg ha$^{-1}$ of zinc sulfate in the condition of no nitrogen utilization increased the SBN by 52% compared to the control. The evaluation of the percentage of ground cover by canopy (GC) confirmed that the main effects of sulfur and nitrogen application on this component were significant ($p<0.01$). The lowest amount of GC was obtained under no nitrogen consumption. Although the use of sulfur-containing fertilizers under no nitrogen consumption increased the amount of this morphological characteristic, the greatest effect of sulfur fertilizers on GC was

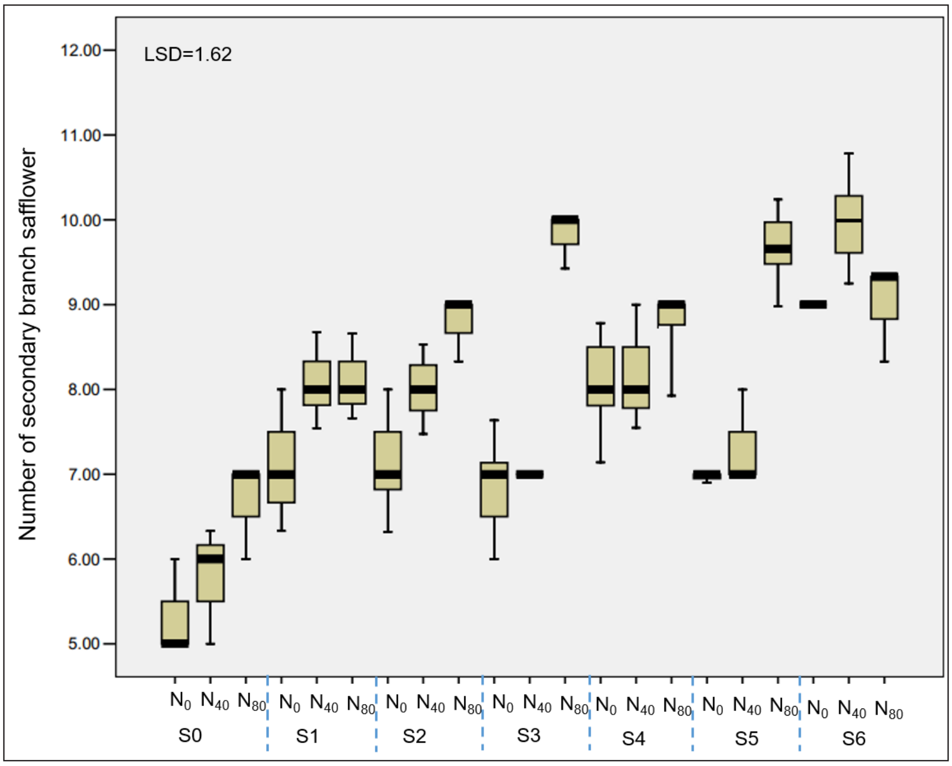


*Fig. 1. The effect of different sulfur fertilizers and nitrogen level on number of secondary branch in safflower grown in west of Iran. The dashed lines in the boxes indicate the means and the means with a difference greater than the LSD have a statistically significant difference.*

recorded for plants grown by consumption of large amounts of nitrogen (Table 1).

Mean comparisons of biological yield (BYM) showed that nitrogen consumption significantly increased the amount of this trait ($p<0.01$). The lowest biological yield was recorded in the condition of not consuming nitrogen and sulfur. The comparison of mean BYM between nitrogen levels showed that there is no significant difference between the control and consumption of 40 kg, while the consumption of 80 kg $ha^{-1}$ of nitrogen increased this trait by about 15% over the control. Consumption of 50 kg of sulfur phosphate, 25 and 50 kg $ha^{-1}$ of elemental sulfur, and 50 kg $ha^{-1}$ of zinc sulfate along with the application of 80 kg $ha^{-1}$ nitrogen produced the highest biological yield. Application of $S_2N_{80}$, $S_3N_{80}$, $S_4N_{80}$ and $S_6N_{80}$ increased the biological yield by 47%, 34%, 30%, and 31% over the control (Table 1). Examining the chlorophyll content of the upper leaves confirmed that nitrogen application greatly increased this photosynthetic pigment. Utilization of 40 and 80 kg $ha^{-1}$ of nitrogen increased the amount of chlorophyll by 10% and 24% compared to no nitrogen consumption condition. However, the highest amount of chlorophyll was recorded under simultaneous application of 50 kg $ha^{-1}$ zinc sulfate or 50 kg $ha^{-1}$ of elemental sulfur and 80 kg of nitrogen (Fig. 2), which was 43% higher than the control condition ($N_0S_0$). Even though the number of shriveled seeds was not significantly affected by nitrogen and sulfur, means comparison indicated that the percentage of unfilled seeds was higher in the conditions of no nitrogen and no sulfur consumption than in other conditions. The lowest amount of wrinkling was recorded in the condition of $N_{80}S_6$, which was about 30% less than the control condition. The results of our experiment showed that the application of high levels of nitrogen and some sulfur-containing fertilizers such as zinc sulfate and elemental sulfur had synergistic effects. So that they strengthened their improving effects on the evaluated traits. The effects of nitrogen on most of evaluated traits were significant except the percentage of shriveled seeds, harvest index and number of heads, which indicates that nitrogen deficiency is evident in the soil of the studied area. A similar trend was observed for the effects of sulfur, which probably indicates that the soil of the region faces some serious limitations in terms of chemical properties. It

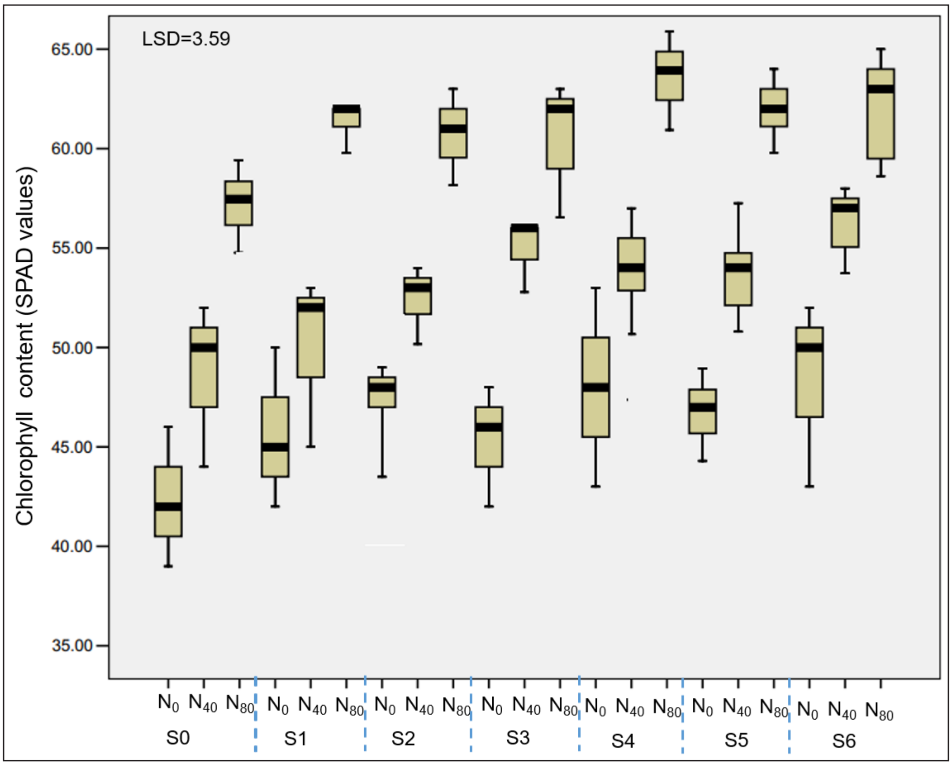


*Fig. 2. Chlorophyll content of upper leaves (SPAD value) in safflower grown under different sulfur and nitrogen level in west of Iran.*

*Table 2 The effect of application of different levels of nitrogen and sulfur fertilizers on safflower yield components in semi-arid region.*

| | | CD | CNP | TSW | SPP | PSY | HI |
|---|---|---|---|---|---|---|---|
| $N_0$ | $S_0$ | 19.7 ± 0.29 | 10.3 ± 0.76 | 26.7 ± 0.76 | 422.7 ± 55.26 | 10.5 ± 1.75 | 14.71 ± 0.77 |
| | $S_1$ | 21.3 ± 0.29 | 11.3 ± 1.00 | 28.7 ± 0.29 | 565.3 ± 74.00 | 20.6 ± 4.69 | 14.10 ± 0.68 |
| | $S_2$ | 20.3 ± 0.76 | 13.3 ± 1.00 | 30.0 ± 0.13 | 609.3 ± 66.33 | 19.7 ± 2.38 | 14.36 ± 0.06 |
| | $S_3$ | 22.3 ± 0.58 | 16.0 ± 1.26 | 29.1 ± 0.40 | 452.7 ± 47.65 | 13.6 ± 2.88 | 16.79 ± 0.82 |
| | $S_4$ | 22.0 ± 1.32 | 12.3 ± 1.04 | 29.1 ± 1.00 | 633.0 ± 62.96 | 21.2 ± 3.03 | 17.91 ± 0.85 |
| | $S_5$ | 23.3 ± 1.15 | 12.7 ± 0.00 | 29.3 ± 0.63 | 596.3 ± 66.66 | 15.1 ± 3.68 | 18.72 ± 0.86 |
| | $S_6$ | 24.0 ± 0.00 | 14.0 ± 1.32 | 29.6 ± 0.32 | 669.3 ± 52.79 | 25.8 ± 5.09 | 19.98 ± 0.72 |
| $N_{40}$ | $S_0$ | 21.0 ± 0.50 | 11.7 ± 1.26 | 27.1 ± 1.00 | 418.0 ± 19.40 | 11.6 ± 0.87 | 15.14 ± 1.04 |
| | $S_1$ | 23.3 ± 0.29 | 12.3 ± 1.53 | 28.5 ± 0.75 | 534.0 ± 31.51 | 18.1 ± 0.66 | 16.98 ± 1.60 |
| | $S_2$ | 22.7 ± 0.76 | 13.7 ± 1.53 | 28.7 ± 0.58 | 604.3 ± 49.01 | 18.7 ± 3.44 | 16.43 ± 0.04 |
| | $S_3$ | 24.3 ± 0.76 | 12.3 ± 2.50 | 28.3 ± 0.15 | 583.0 ± 57.16 | 21.2 ± 5.25 | 15.52 ± 0.26 |
| | $S_4$ | 25.0 ± 0.50 | 15.0 ± 0.76 | 29.4 ± 0.28 | 793.3 ± 18.89 | 26.9 ± 4.01 | 18.77 ± 1.22 |
| | $S_5$ | 23.0 ± 0.87 | 13.0 ± 1.73 | 29.2 ± 0.39 | 743.3 ± 49.48 | 28.1 ± 4.54 | 18.43 ± 0.23 |
| | $S_6$ | 23.3 ± 0.29 | 14.7 ± 0.50 | 29.7 ± 0.55 | 737.7 ± 12.24 | 27.7 ± 3.78 | 19.57 ± 0.31 |
| $N_{80}$ | $S_0$ | 22.7 ± 0.29 | 11.0 ± 1.32 | 28.3 ± 0.29 | 397.3 ± 2.89 | 10.9 ± 0.99 | 14.52 ± 0.97 |
| | $S_1$ | 25.0 ± 0.87 | 12.0 ± 1.32 | 29.7 ± 0.23 | 621.7 ± 75.56 | 24.6 ± 5.09 | 16.14 ± 1.63 |
| | $S_2$ | 26.3 ± 0.29 | 11.7 ± 0.58 | 30.3 ± 0.76 | 661.7 ± 49.29 | 24.0 ± 1.56 | 14.77 ± 0.68 |
| | $S_3$ | 26.3 ± 0.76 | 13.0 ± 2.18 | 30.1 ± 0.38 | 635.7 ± 20.80 | 22.3 ± 2.62 | 15.33 ± 0.64 |
| | $S_4$ | 25.3 ± 0.76 | 14.3 ± 2.08 | 30.2 ± 0.13 | 679.0 ± 71.39 | 24.6 ± 3.40 | 17.95 ± 1.61 |
| | $S_5$ | 27.0 ± 0.87 | 15.7 ± 1.04 | 31.3 ± 0.43 | 706.7 ± 58.27 | 27.7 ± 6.07 | 17.29 ± 0.42 |
| | $S_6$ | 27.0 ± 1.00 | 15.0 ± 1.61 | 30.4 ± 0.20 | 422.7 ± 55.26 | 33.56 ± 3.53 | 18.35 ± 0.47 |
| LSD | | 2.36 | 2.21 | 1.57 | 176.36 | 4.32 | 2.40 |
| Significance level | | | | | | | |
| N | | 0.001 | 0.572 | 0.001 | 0.046 | 0.014 | 0.125 |
| S | | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 | 0.001 |
| N×S | | 0.330 | 0.006 | 0.899 | 0.722 | 0.046 | 0.497 |

Note: $N_0$: no nitrogen application, $N_{40}$: application of 40 kg ha$^{-1}$ nitrogen, $N_{80}$: kg ha$^{-1}$ nitrogen, S0: control, S1 and S2: 25 and 50 kg ha$^{-1}$ S via sulfur phosphate composites, S3 and S4- 25 and 50 kg ha ha$^{-1}$ of S via elemental sulfur, S5 and S6: 25 and 50 kg ha$^{1}$ of S via zinc sulfate. The numbers represent the mean ± standard deviation. CNP: capitulum number per plants, PSY: plant seed yield, STSW: the weight of thousand seeds, PH: plant height, CD: the diameter of the capitolum, BYM: biological yield, SD: stem diameter, SS: percentage of shriveled seeds. At the significance level P value are presented the numbers smaller than one and five are significant at the statistical level of one and five percent, respectively. Numbers greater than 5 indicate that the mentioned treatment is statistically insignificant. If the difference between two means in each column is more than the LSD, then those means differ significantly.

seems that the number of unfilled and shriveled seeds is partly under genetic control and less affected by fertilizer management (Koutroubas and Papakosta, 2010). In the mentioned area, the water deficit during the grain filling periods is one of the factors in reducing the rate of seed filling, and probably increasing the amount of available water can improve seed filling.

The application of nitrogen and sulfur significantly increased the diameter of the capitulum ($p < 0.01$). Specifically, the use of 40 and 80 kg kg$^{-1}$ of nitrogen enhanced this component by 6% and 17%, respectively, compared to the control. The most significant effect of sulfur fertilizers was observed in plants grown with 50 kg ha$^{-1}$ of zinc sulfate, which resulted in an 18% increase in the capitulum diameter (Table 2). However, the application of 25 kg kg$^{-1}$ of zinc sulfate and 50 and 25 kg kg$^{-1}$ of elemental sulfur also improved the diameter of the capitulum, with results closely comparable to those of S6, though differences were not statistically significant. The lowest canopy width in plants grown with sulfur fertilizers was related to the application of 25 kg ha$^{-1}$ of sulfur phosphate, which was 27%

higher than the control conditions. While the highest canopy width was recorded for plants grown by 50 kg $ha^{-1}$ of zinc sulfate, which was 48% higher than the control condition (Fig. 3). The synergistic effects between nitrogen and sulfur on this trait were more noticeable under the conditions of application of high levels of elemental sulfur and zinc sulfate. Assessment of the number of capitulum per plant (CNP) showed that the use of sulfur-containing fertilizers under no nitrogen consumption conditions did not have considerable effect on the improvement of the CNP, while the combined use of 40 or 80 kg $ha^{-1}$ of nitrogen along with sulfur fertilizers especially $S_4$, $S_5$ and $S_6$, increased this key component of seed yield (Table 2). The results showed that thousand seed weight (TSW) was affected by nitrogen application ($p<0.01$), However, only the application of high levels of nitrogen increased TSW compared to the control (4%). Seed weight was affected by sulfur application ($p<0.01$), and the heaviest seeds were obtained by applying different levels of zinc sulfate and 50 kg $ha^{-1}$ of sulfur phosphate, which were about 9% heavier than the seeds grown under control conditions. These results were not far-fetched because sulfur is present in many key biomolecules such as biotin and thiamine and also it plays a role as a structural and effective part in S-*Adenosyl methionine* (SAM) and sulfoquinovosyl diacylglycerol (SQDG) which is concentrated in the inner membrane of *chloroplast* (Jamal *et al.*, 2010). However, the mutual effects of nitrogen and sulfur were non-significant in some traits, which is probably caused by some compensatory effects of changes in other traits or the need to consume more nitrogen or sulfur to cause a significant response. The presence of synergistic effects between nitrogen and zinc sulfate or elemental sulfur was consistent with the results of Fazili *et al.* (2008) they also observed a significant interaction between nitrogen and sulfur and stated that any nutritional imbalance in wheat crop can increase the amount significantly reduce performance. Sulfur also plays a critical role in cell division, so it can directly affect plant seed weight. Mean comparison of number of seeds per plant (SPP) revealed that on average, the application of sulfur increased the SPP by 53% compared to the control condition (no sulfur application), and the highest increase was recorded for plants

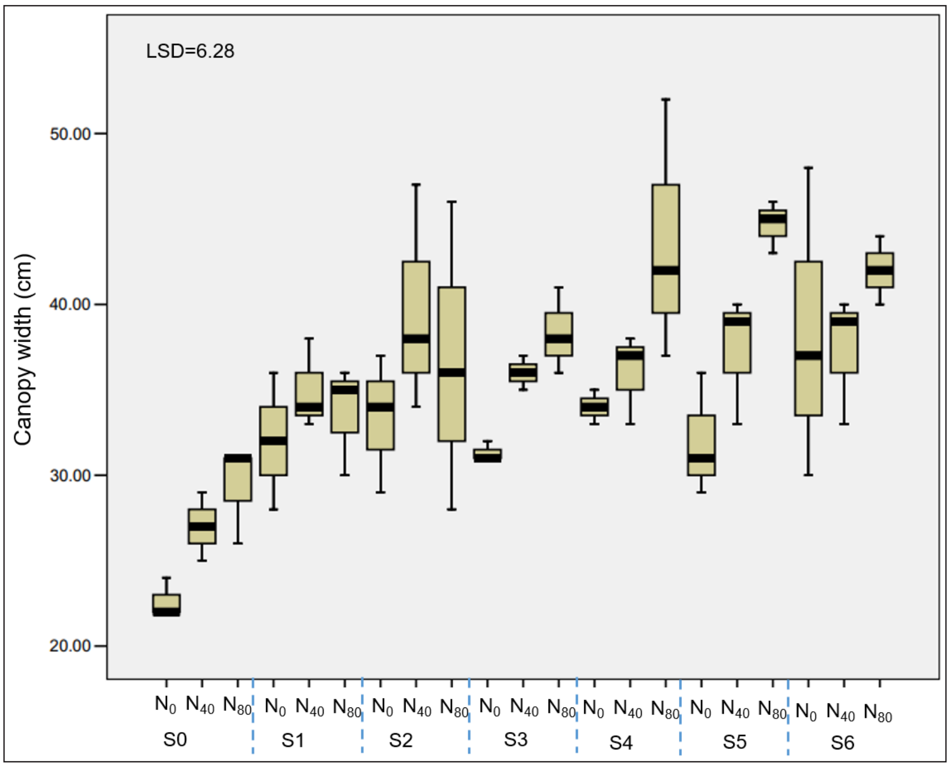


*Fig. 3. The effect of application of different types of sulfur fertilizers and different levels of nitrogen on safflower canopy growth under field conditions in the western region of Iran*

grown by the application of 50 kg ha$^{-1}$ of zinc sulfate, with 78% increase over the control (Table 2). These researchers suggested that to obtain the maximum efficiency of nitrogenous fertilizers, it seems necessary to use sulfur in the conditions of semi-arid regions. Also, in line with the results obtained in our experiment, the sole and combined applications of nitrogen (N) and sulfur with variable application rates significantly affect the plant height, grains number per spike, spike number in unite area, and grain weight. The addition of 40-120 kg ha$^{-1}$ nitrogen significantly improved seed yield when compared with plants grown under no nitrogen and no sulfur application conditions (Dawar *et al.*, 2022). When the plant is under environmental stress conditions such as water shortage, organic compounds called osmolytes are produced inside the plant, which increases the plant's ability to deal with stress conditions. *Thibobacillus* and sulfur bacteria play an important role in increasing tolerance to irrigation stress by increasing the activity of oxidizing enzymes and increasing the content of osmolytes. Therefore, it can be said that sulfur plays an important role in improving the quantitative and qualitative performance of plants (Mustafa *et al.,* 2022).

Examining the dry weight of the plant showed that the application of nitrogen under no sulfur condition had little effect on this component. However, the use of nitrogen, especially its large amounts along with sulfur fertilizers, increased the dry weight of the plant between 25 to 84% (Fig. 4). Despite the low effects of sulfur phosphate on other components, its synergistic effects with nitrogen on plant dry weight were evident. The highest dry weight of the plants was related to the plants grown with the application of 50 kg ha$^{-1}$ of zinc sulfate and 80 kg ha$^{-1}$ of nitrogen. The results of soil analysis indicated that the soil of the studied area was facing limitations in terms of physical and chemical conditions. On the other hand, according to the climate of the region, semi-arid climate, and insufficient rainfall have caused the accumulation of carbonates in the soil, and one of the problems that most farmers face is the limitations of cultivation in calcareous soils. Lime dissolves in acidic to neutral pH soils, but does not dissolve in alkaline soils and acts as a reservoir for phosphate precipitation. The main problem of these soils is that the amount of phosphorus and organic matter in calcareous soils is low, and the high pH of the soil reduces the availability of micronutrients such as zinc and iron as well as phosphorus (Naorem *et al.,*

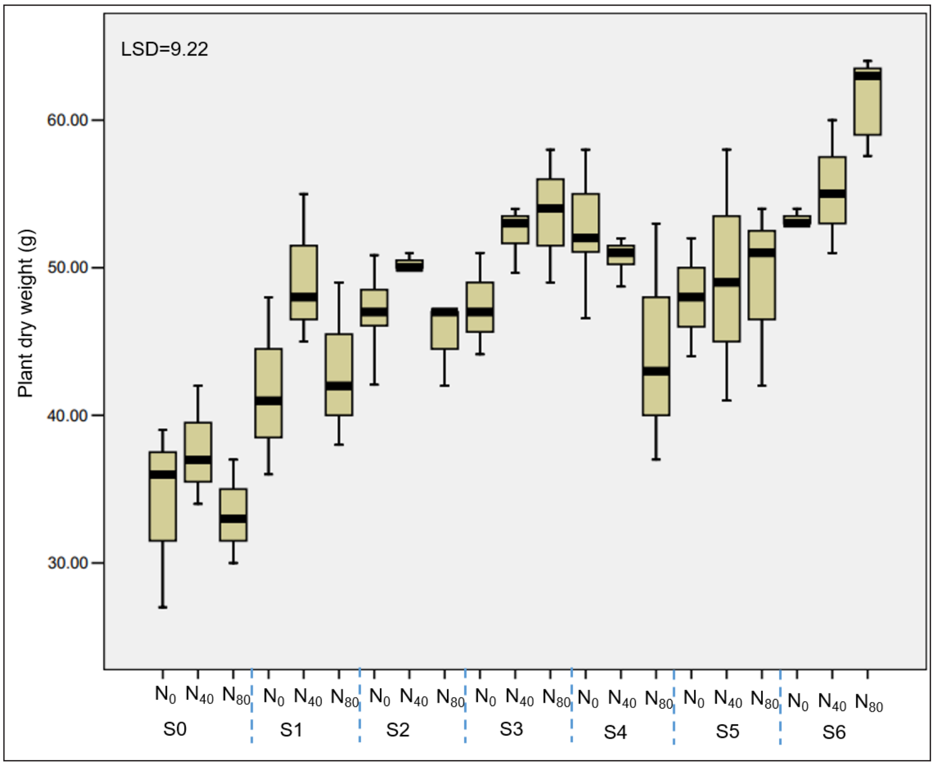


*Fig. 4. The effect of different sulfur fertilizers and nitrogen level on safflower dry weight grown in west of Iran.*

2023). It seems that this is also the reason for the extraordinary effectiveness of zinc sulfate compared to other sulfur fertilizers.

Investigation of the harvest index confirmed that the effect of sulfur application on this index was significant. However, the mean comparison showed that the application of $S_6$, $S_4$, and $S_5$ increased the harvest index by 30, 23 and 22%, respectively. No significant difference was observed between other application levels of sulfur and the control. The measurement of seed yield indicated the presence of significant mutual effects of nitrogen and sulfur. Application of 40 and 80 kg ha$^{-1}$ nitrogen increased seed yield by 5% and 12%. However, the effects of nitrogen application were more noticeable under conditions of application of elemental sulfur and zinc sulfate. The highest yield was recorded for plants grown under the application of $S_6$ and $S_4$ conditions with values of 1366 and 1326 kg ha$^{-1}$, respectively, which was a significant increase compared to the control condition with 954 kg ha$^{-1}$ seed production (Fig. 5). The results of our experiment showed that the application of high levels of nitrogen and zinc sulfate or elemental sulfur affected the source and sink relationship. An increase in the number of secondary branches, chlorophyll content, width of the canopy, a dry weight of the plant all indicate an increase in the size of the photoassimilates supplier sources. On the other hand, the improvement of yield components and seed production under simultaneous use of nitrogen and sulfur fertilizers indicated the improvement of the activity or the size of the sinks, or the better distribution of photoassimilates on the plant organs, which has led to improved final seed yield.

Principal component analysis is used to explain the correlation between the traits and also how the combined treatments affect the measured traits. Plots of the first two PCAs showed that the first principal component separated the efficient levels of nitrogen fertilizer (Fig. 6). PCA could separate the sulfur fertilizer by the second component so that effective levels of sulfur fertilizer i.e., $S_6$, $S_4$, $S_5$, and $S_3$. Also, PCA provided the correlation coefficient between any two traits by the cosine of the angle between their vectors of related traits. A strong positive association between seed yield, a positive correlation was observed between the seed yield and width of the canopy,

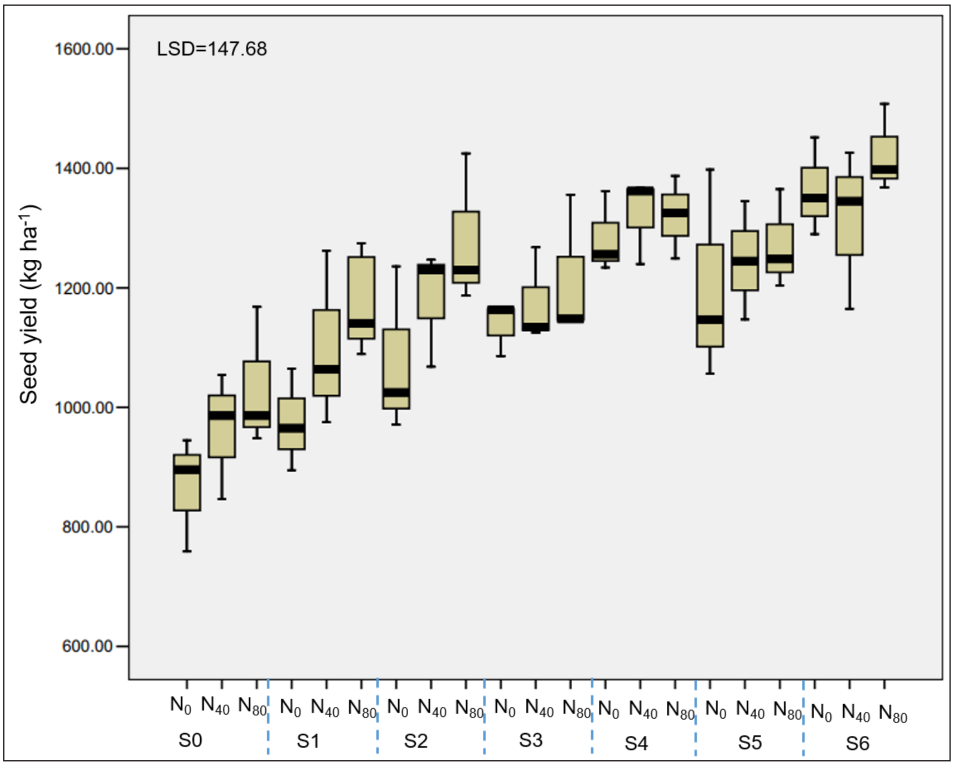


*Fig. 5. The effect of application of different types of sulfur fertilizers and nitrogen levels on safflower seed yield growth under irrigated field conditions in the western region of Iran.*

the number of seeds per plant, the number of seeds in the capitulum, and the weight of 1000 seeds, due to the closeness of the vector of mentioned trait in the plot (r = cos 0 = +1).

The use of materials such as gypsum, sulfuric acid, and sulfur can be useful in improving calcareous soils. Sulfur is one of the most common and economical soil amendment materials that is used to acidify the soil today. However, the effect of sulfur on acidifying the soil and increasing the ability to absorb nutrients depends on the amount of sulfur oxidation and sulfuric acid production (Abdin *et al.*, 2003). The biological oxidation of sulfur in the soil is mainly carried out by bacteria of the *Thiobacillus* genus, their sufficient activity can supply the sulfate needed by the plants, and will result in a local decrease in soil pH rhizosphere and an increase in the absorption of elements such as phosphorus, zinc, iron, and other micronutrients. The release of the high-yielding varieties of crops significant changes in the consumption of traditional fertilizers containing sulfur (ammonium sulfate, single superphosphate), and changes in the amount of coal fuel consumption and the reduction of acid rain are all among the things that have reduced the amount of sulfur in the soil (Hinckley *et al.,* 2020). It appears that the sulfur deficiency in the soil causes a significant decrease in the amount of nitrogen absorption and negatively affects the efficiency of the root system, and most of the nitrogen is removed from the reach of the roots through leaching (Grzebisz *et al.,* 2022). Mehmood *et al.* (2021) reported that the consumption of 40 kg ha$^{-1}$ sulfur in sesame plants increased nitrogen absorption by 13%, and compared to control conditions ($S_0$), the amount of nitrogen in leaves, seeds and capsules increased. Taken together, obtained results indicated that the balanced use of nitrogenous and sulfur fertilizers is necessary

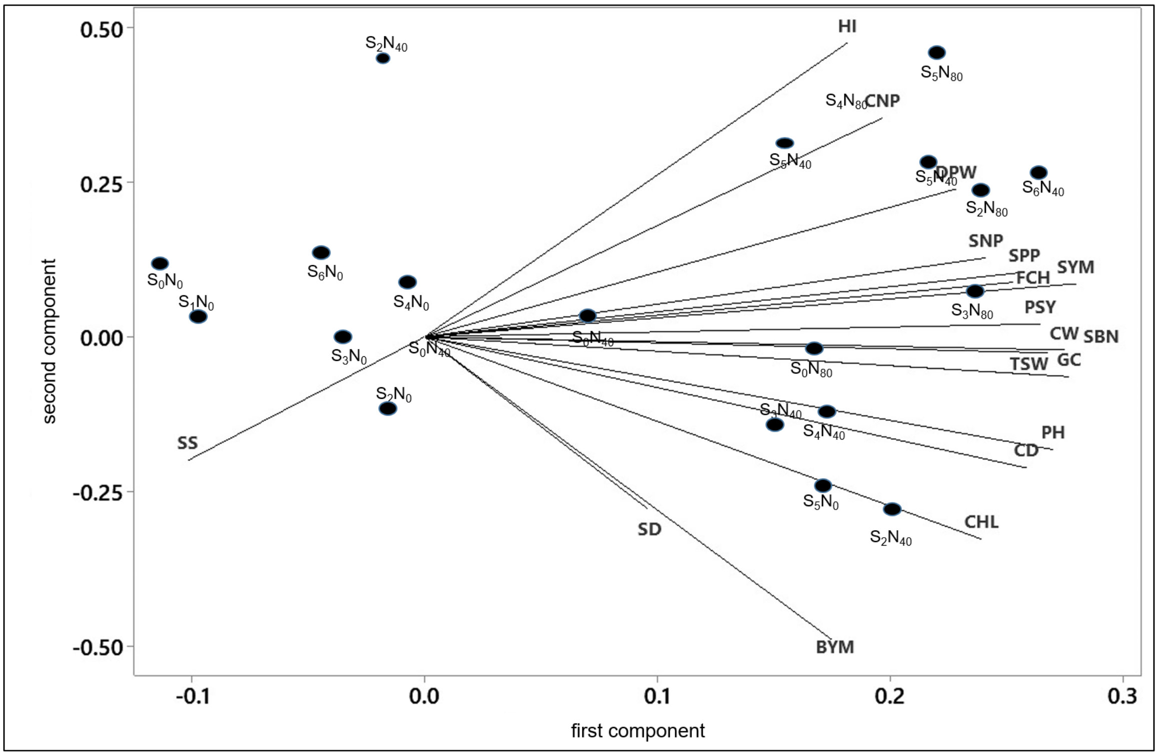


*Fig. 6. Plot of the first two PCAs showing relation among various agronomical traits of safflower grown under different levels of sulfur. HI: harvest index, CNP: capitulum number per plants, DPW: dry plant weight, SNP: number of the seed in main capitulum, SPP: number of the seed in plant, The height of the first capitulum from the ground level, PSY: plant seed yield, CW: canopy width, SBN: number of the secondary branch, GC: ground cover percentage, TSW: the weight of thousand seeds, PH: plant height, CD: the diameter of the capitolum, CHL: Chlorophyll content, BYM: biological yield, SD: stem diameter, SS: percentage of shriveled seeds. $S_0$- control (no sulfur application), $S_1$- 25 kg ha$^{-1}$ of sulfur through the sulfur phosphate composites, $S_2$- 50 kg ha$^{-1}$ of sulfur through the phosphate composites, $S_3$- 25 kg ha$^{-1}$ of sulfur through the elemental sulfur, $S_4$- 50 kg ha$^{-1}$ of sulfur through elemental sulfur, $S_5$- 25 kg ha$^{-1}$ sulfur through zinc sulfate, $S_6$- 50 kg ha$^{-1}$ sulfur through zinc sulfate. $N_0$ = no nitrogen fertilizer application, $N_{40}$ = 40 and $N_{80}$ = 80 kg ha$^{-1}$ nitrogen fertilizer application.*

to improve vegetative growth and increase the amount of seed production and possibly seed oil content. In the studied area, the best results were achieved with the application of high levels of zinc sulfate and the consumption of 80 kg $ha^{-1}$ nitrogen, which confirms the findings of Dawar *et al.* (2022).

## Conclusions

The outcomes of this experiment corroborated that the application of nitrogen and sulfur fertilizers alone increased the growth and yield components of safflower. However, their combined application resulted in synergistic effects and increased the positive effect of fertilizers. The increase in the number of lateral branches, the number of the capitulum, and the width of the canopy were evident under the combined application of nitrogen with zinc sulfate or elemental sulfur. Our finding showed that for improving the effectiveness of nitrogen fertilizer soil amendment by the application of sulfur fertilizers (especially zinc sulfate or elemental sulfur) seems to be an essential agronomic management.

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