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Research article

Evaluation of Sulfur and Nitrogen Utilization on Agronomic Traits and Fatty Acid Profiles of Safflower Using a Tester Biplot Model

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Abstract

This study investigated the effects of different sulfur fertilizers and nitrogen levels on the agronomic performance and fatty acid profile of safflower. The experimental treatments included sulfur application at varying rates and sources: S0 (no sulfur application), S25 (25 kg ha^{-1} sulfur from elemental sulfur, ES), S50 (50 kg ha^{-1} sulfur from ES), ZS25 (25 kg ha^{-1} sulfur from zinc sulfate, ZS), and ZS50 (50 kg ha^{-1} sulfur from ZS). Nitrogen was applied at three levels: N0, N40, and N80 (0, 40, and 80 kg ha^{-1} nitrogen from urea fertilizer). The experiment was conducted in Baneh, Iran, in 2021. The entry-by-tester (treatment-by-trait) biplot analysis, which accounted for 80% of the observed variability, identified the N80-ZS50 treatment as the most effective in enhancing key traits, including yield, oil content, and specific fatty acids such as linolenic acid. Additionally, other unsaturated fatty acids, including oleic, linoleic, and arachidic acids, exhibited higher concentrations under the N0-S50 treatment. A positive correlation was observed between fatty acid composition, oil content, and protein, as well as among various agronomic traits. Based on overall performance and trait differentiation, N80-ZS50 emerged as the optimal treatment, followed by N80-S25. Trait discriminative analysis highlighted stearic acid, oil content, and linolenic acid as key determinants in safflower evaluation. These findings underscore the significant influence of sulfur and nitrogen fertilization on safflower characteristics, demonstrating the benefits of their combined application. The N80-ZS50 treatment (80 kg ha^{-1} nitrogen and 50 kg ha^{-1} sulfur from zinc sulfate) is recommended to enhance safflower performance in upland semi-arid regions.

Keywords: Ideal trait; ideal treatment; pentagon shape; vector view

1. Introduction

Following cereal crops, oilseeds are the second important source of enteric foods in mankind and livestock nutrition and among the oilseed crops, safflower provides a lot in terms of seed oil quality and medicinal properties. Safflower (*Carthamus tinctorius* L.) is one of the old-world crops with a history of cultivation of about 4000 years in the world. It

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is also a crop that is widely adaptable to different regions (Cheng et al., 2024). It is produced in almost 18 countries. Its cultivated area in the world in 2022 was 1.12 million ha, and its seed production was 948 kg ha^{-1}. The area under safflower cultivation in Iran was equal to 36500 ha, with a mean performance of 1325 kg ha^{-1}. However, according to the high yielding record, countries such as Mexico (2000 kg ha^{-1}), Tajikistan (1600 kg ha^{-1}), China (1500 kg ha^{-1}), and the United States (1400 kg ha^{-1}), have the highest sunflower yield in the world (Rostami et al., 2023). Safflower has valuable characteristics of compatibility with arid and semi-arid climatic conditions. Furthermore, it is of high oil quality, and tolerance to abiotic stress. It also has spring and autumn types. The superior quality of the oil and greater tolerance to adverse environmental conditions such as dryness and salinity have led to numerous studies on safflower (Rahnama et al., 2024). In arid and semi-arid areas, soil organic matter is a natural source of nitrogen required by the plant and the presence of stressors decreases its availability. In such regions, water stress is the main obstacle to nitrogen absorption; therefore, under moisture stress, nitrogen shortage becomes the main factor restricting yield productivity. Nitrogen is important for crop growth and development, and attention should be paid to its amount and management of its use during the plant growth cycle (Zanetti et al., 2022). It is an important and vital element for plants, which is present in the structure of proteins, nucleic acids and chlorophyll. Application of a sufficient amount of nitrogen and its availability in sensitive phenological stages of plant growth can increase crop yield (Manvelian et al., 2021). Thus, it should be kept in mind that high nitrogen application may affect the partitioning of photoassimilates, the ratio of carbohydrates to protein, and the proportion of oil in the achene.

Sulfur is one of the essential nutrients for all living organisms, and it is used as an amendment to reduce soil pH. It is widely used in arid and semi-arid environments to improve the properties of sodic and saline-sodic soils and to increase the absorption of low-use nutrients such as zinc, iron and phosphorus. The optimal ratio of nitrogen to sulfur concentration in plant systems is about 10-15, but in oilseed plants, sulfur is involved in various processes of oil biosynthesis, and hence an optimal ratio of N to S below 10 can improve the quantity and quality of oil production (Tian et al., 2020). Sulfur is used to make proteins and enzymes by participating in the building of amino acids, methionine and cysteine. Also, sulfur is involved in the synthesis of chlorophyll, vitamins thiamine and biotin, glutamine and coenzyme A and increases plant resistance to drought stress conditions. Sulfur protects plants from diseases, drought and cold and prevents the accumulation of nitrates in plant tissues (Fatma et al., 2016). Application of sulfur increases dry matter production and improves yield components of safflower like high capitula per unit area (Janmohammadi et al., 2017). Considering the importance of safflower as an oilseed crop, as well as calcareousness and high acidity of Iran's soils, and the unaviailability of many nutreints at high pH, the need to use sulfur with the aim of increasing oil production in the plant and improving the soil condition is obvious.

The interaction of elements on the growth and performance of field crops is important, especially between nitrogen and sulfur elements, which have a main influence in the life cycle of safflower plants. The interaction of sulfur and nitrogen is important in creating a balance for sulfur through their influence on photosynthesis processes, carbohydrate production, and allocation of photoassimilates to oil or protein biosynthesis pathways. Kulczycki (2021) reported a positive interaction between nitrogen and sulfur sulfate on grain yield, oil percentage and absorption of nutrients in rapeseed and winter wheat (Kulczycki, 2021). Considering the high solubility of nitrogenous fertilizers and the limited development of the roots of agricultural plants at the beginning of the planting time, it is necessary to carry out accurate experiments in relation to the appropriate amount of consumption of these fertilizers in different agricultural plants in order to prevent the excessive use of chemical fertilizers and to increase fertilizer use efficiency. Considering the impact of nitrogen application on key processes such as photosynthesis and the possibility of interaction between N and S, it is necessary to improve the absorption and effect of sulfur by optimally supplying nitrogen. Thus, this research was performed to investigate the effect of sulfur fertilizer consumption at different levels of nitrogen on safflower agronomic traits and fatty acid profile.

2. Materials and Methods

Research was conducted in a split-plot layout based on a randomized complete block design with three replicates, in Baneh (35°59′N, 45°53′E), Iran, during the 2021 growing season which had 150 mm of rainfall across the growing period. The soil texture was sandy clay loam, and some properties of the soil are given in Table 1. The main plot was sulfur as S1 (no usage), S25 (25 kg ha^{-1} sulfur from elemental sulfur fertilizer, ES), S50 (50 kg ha^{-1} sulfur from ES), Zs25 (25 kg ha^{-1} sulfur from zinc sulfate, ZS), Zs50 (50 kg ha^{-1} sulfur from ZS). The sub-plots were nitrogen usage as N0, N40 and N80 (no application of nitrogen and utilization of 40 and 80 kg ha^{-1} nitrogen from urea fertilizer, respectively). The plot size was 16 m^{-2} (8 rows with 4 m in length). Sulfur treatments were applied before sowing while nitrogen treatments were used at the sowing, stem elongation and capitulum presence. Variety ZY-S, a widely cultivated variety in Iran, was planted by hand in April at a depth of 3 cm, with 50 cm inter-row and 10 cm intra-row spacing. Irrigation was performed after sowing and weeding was done manually.

Table 1. The results of the soil physico-chemical analysis of the studied field

pH	OC (%)	TN (%)	$CaCo_3$ (%)	EC (ds m^{-1})	Fe (ppm)	Mn (ppm)	P (ppm)	K (ppm)
7.68	0.51	0.26	17	2.15	1.5	7.09	16.28	625

OC: organic carbon, TN: total nitrogen, EC: electrical conductivity

From ten randomly selected plants from each experimental unit, plant height (PH) was recorded. Seed yield (SY) was evaluated by harvesting the middle six rows of plants in each experimental plot (9 m^{-2}) and threshing and separating the seeds. From the total harvested seeds in each experimental plot, a random cup of seeds was selected for measuring the thousand seeds weight (TSW). The chlorophyll content (CHL) was recorded using a SPAD-02 Plus chlorophyll meter (Konica Minolta Optics, Japan). Also, oil percent (OIL), ash percent (ASH) and protein content (PRO) were measured. Oil extraction was performed by mixing seed samples with hexane at a ratio of 1:5 and stirring for 24h. For the methyl ester process, 50 mL was poured into a tube (with a lid) that contained 1 mL of hexane, and the contents were shaken with 100 µL of sodium methoxide in methanol (30% solution) (Ortega et al., 2008). The hexane layer was removed, and sodium sulfate was used to eliminate the moisture. For injection into gas chromatography (Agilent 6890N, USA), 1 µL of the hexane phase was gathered. It was supplied with a FFAP-TC capillary column and an FID detector, and nitrogen was the carrier gas used to obtain the fatty acids profile as oleic acid 18:1 (OLE), stearic acid 18:0 (STE), palmitic acid 16:0 (PAL), linoleic acid 18:2 (LINL), linonenic acid 18:3 (LINN) and arachidic acid 20:0 (ARA).

The data were subjected to analysis using the tester biplot model (Yan, 2019) via the GGEbiplot software. Although the genotype (G) plus genotype by environment (GE) interaction model as GGE (G+GE) is used for stability analysis, this method can be used for any two-way layouts like treatment by trait (TT) interactions (Yan, 2019). This model can be effectively adapted for treatment-by-trait evaluation by treating treatments as genotypes and traits as testers. This approach enables researchers to visualize which treatments perform best for specific traits, identify trait correlations, and detect key discriminative traits, ultimately guiding treatment recommendations. This method simplifies complex relationships, aiding optimal treatment selection and trait prioritization in agronomic and quality trait studies. However, the TT biplot model shows graphic grasp from interaction structure of measured traits (testers) across treatments (entries) as:

$$\frac{a_{ij} - \overline{b_j}}{S_j} = \sum_{n=1}^{2} \alpha_n \beta_{in} \gamma_{jn} + \xi_{ij}$$

Where a_{ij} is the mean of each treatment or entry i for each trait or tester j, $\overline{b_j}$ is the mean of entries for tester j, S_j is the standard deviation of tester j for entries, α_n is the eigenvalue for principal component, PC n, β_{in} and γ_{jn} are values for entry i and tester j on PC n, and ξ_{ij} is the residual term of the fitted equation related to treatment i for trait j. Also, for obtaining symmetrical scales of testers and entries, the eigenvalue is corrected through vector absorption, so a normal presentation of testers and entries can happen. The entry by tester interaction biplots were generated by these symmetric scales, and each entry or tester is shown by a special sign.

3. Results and Discussion

The first and second PCs explained 80% of the variability (Figure 1); the first PC contributed 58%, and the second PC contributed 22% to the explained variance. This described variability of the entry by tester interactions demonstrated the important role of additive and crossover types of interactions, so ranks of treatments across traits were different, which is in accordance with previous findings for sunflower (Sabaghnia & Janmohammadi, 2023). These findings indicate that the effects of entry with tester interaction should be considered in interpretations and in this context the use of the biplot method is useful and recommended.

It was previously reported that the biplot model provided an effective method of exploring treatments and traits (Sabaghnia et al., 2024). Figure 1 shows the visual response of nitrogen and sulfur fertilizers and indicates which treatments were better than others in target traits. Most traits, including agronomic traits like PH, SY, CHL, and TSW; and some fatty acid profile consisting of STE, PAL, and LINN acids, as well as oil percent (OIL) and protein content (PRO), were grouped in the section of N80-Zs50. The N0-S50 was the best treatment for obtaining high levels of OLE, LINL, and ARA acids. Finally, N0-S0 indicated high amounts for ash percent (ASH) while the other remained vertex treatments (N40-S0 and N80-S0) were not the best for the measured traits (Figure 1). Thus, the yield performance of safflower is more related to yield components like thousand seed weight; and unsaturated linonenic acid as well as saturated stearic acid, under usage of 80 kg ha^{-1} nitrogen and 50 kg sulfur from Zs, respectively. It was previously shown that linoleic acid was the important oil component of safflower and the most favorable cultivars had

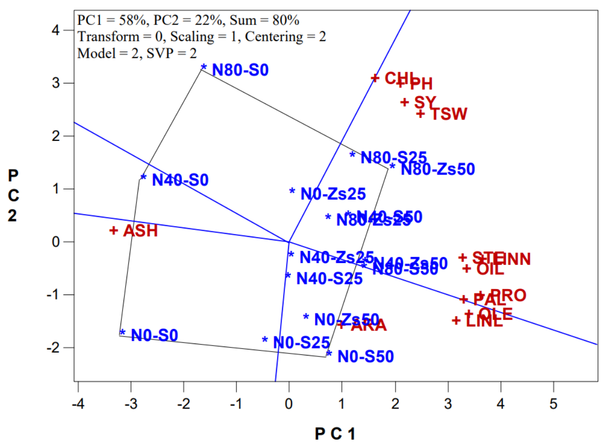

Figure 1. Biplot showing the best treatment combinations and the traits affected by them in safflower under different levels of nitrogen and sulfur application. Treatment combinations are five sulfur levels: S0 (no usage), S25 (25 kg ha^{-1} sulfur from elemental sulfur), S55 (50 kg ha^{-1} sulfur from elemental sulfur), Zs25 (25 kg ha^{-1} sulfur from zinc sulfate), Zs50 (50 kg ha^{-1} zinc sulfate), and three nitrogen levels as N0 (0 kg ha^{-1}), N40 (40 kg ha^{-1}) and N80 (80 kg ha^{-1}). Traits are: PAL, palmitic acid 16:0; STE, stearic acid 18:0; OLE, oleic acid 18:1; LINL, linoleic acid 18:2; ARA, arachidic acid 20:0; LINN linonenic acid 18:3; OIL, oil percent; ash percent (ASH); PH, plant height; SY, seed yield (SY); TSW, thousand seeds weight; and CHL, chlorophyll content.

simultaneously high yield performance and linoleic acid levels (Amirkhiz et al., 2021). However, the entry by tester biplot provided clear insights into the reaction of safflower traits under various fertilizer treatments and facilitated the detection of the best treatment. Thus, for practical advice, application of a high rate of nitrogen and sulfur can be considered for safflower production by farmers of semi-arid regions.

The correlation among testers (Figure 2) indicates the relationships among measured traits of safflower. Among traits where the angle between them is small and sharp, the correlations are positive and significant. Traits are positively associated with angles lower than 90°, negatively correlated with larger than 90°, and no association is indicated with 90° angle (Yan, 2019). Larger trait lines show significance, while shorter vectors indicate no significance and traits at the center position have no significant relation with others (Yan, 2019). Figure 2 indicates that the fatty acid profile, oil and protein were positively related, regarding the small acute angles among them. Additionally, agronomic traits (PH, SY, and TSW) and CHL were positively associated with acute positions. In contrast, there was a negative relation between ASH and fatty acid profile, as demonstrated by the obtuse positions (Figure 2). The relation between ASH and agronomic traits was relatively zero due to their 90° angle as well as the relation between fatty acid profile and agronomic traits. The predictions of entry by tester interaction biplot models for trait relations aligned well with those found in the numerical correlations (Table 2), but there were some minor inconsistencies that slightly reduced the accuracy of the interpretation. It was found that the safflower oil contained unsaturated fatty acids (oleic, linoleic and linonenic) and saturated acid (stearic), which were related positively, making it a desirable source of healthy food oil that could reduce the percentage of undesirable fats such as cholesterol in end consumers (Amirkhiz et al., 2021).

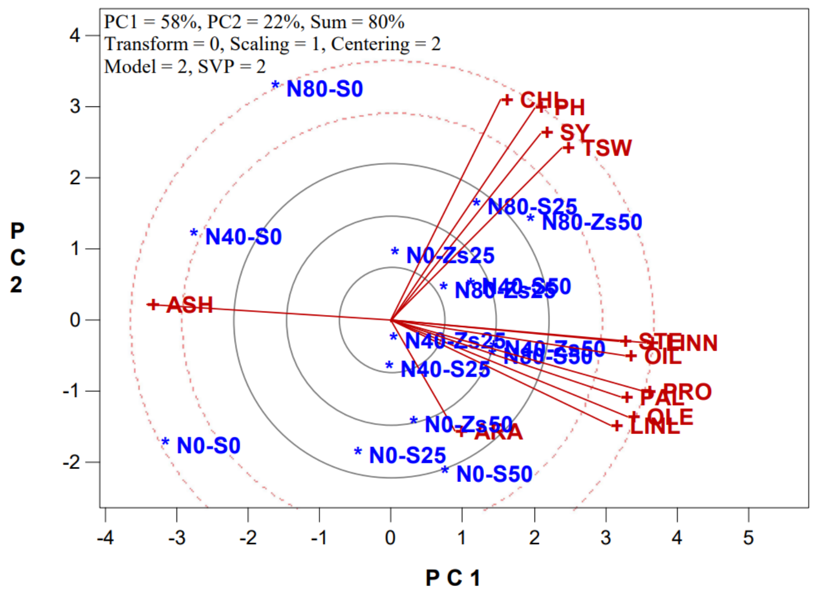

Figure 2. Graphic presentation of interrelationships among measured traits of safflower. Traits are: PAL, palmitic acid 16:0; STE, stearic acid 18:0; OLE, oleic acid 18:1; LINL, linoleic acid 18:2; ARA, arachidic acid 20:0; LINN linonenic acid 18:3; OIL, oil percent; ash percent (ASH); PH, plant height; SY, seed yield (SY); TSW, thousand seeds weight; and CHL, chlorophyll content.

Table 2. Parsons coefficient of correlations among measured traits of safflower

	PH	CHL	TSW	SY	Ash	Oil	Pro	Pal	Ste	Ole	Linl	Ara
CHL	0.93											
TSW	0.72	0.69										
SY	0.84	0.66	0.77									
Ash	-0.33	-0.20	-0.48	-0.40								
Oil	0.37	0.23	0.44	0.42	-0.70							
Pro	0.26	0.14	0.46	0.38	-0.82	0.80						
Pal	0.21	0.06	0.30	0.33	-0.78	0.78	0.86					
Ste	0.34	0.25	0.54	0.45	-0.84	0.63	0.75	0.73				
Ole	0.16	0.07	0.30	0.20	-0.79	0.83	0.86	0.90	0.82			
Linl	0.15	0.06	0.22	0.20	-0.77	0.65	0.81	0.75	0.72	0.78		
Ara	-0.04	-0.05	-0.08	-0.09	-0.28	0.28	0.30	0.18	0.14	0.24	0.46	
Linn	0.37	0.29	0.42	0.38	-0.80	0.86	0.85	0.77	0.70	0.84	0.80	0.39

Critical correlation values, degrees of freedom =19 and P < 0.01 are 0.43 and 0.55, respectively. Traits are: PAL, palmitic acid 16:0; STE, stearic acid 18:0; OLE, oleic acid 18:1; LINL, linoleic acid 18:2; ARA, arachidic acid 20:0; LINN linonenic acid 18:3; OIL, oil percent; ash percent (ASH); PH, plant height; SY, seed yield (SY); TSW, thousand seeds weight; and CHL, chlorophyll content

According to correlation coefficients (Table 2), seed yield had positive correlation with yield components like number of capitula per plant, seeds per plant and thousand seed weight but it did not show any relation with fatty acid profile except for stearic acid. Also, oil percent had positive associations with seed yield performance and some yield components like thousand seed weight, while it indicated a high positive relation with all fatty acid profile except arachidic acid (Table 2). Relatively good positive relations were observed among morphological traits as well as among components of fatty acid profile.

The distinction potential of a treatment for traits and its typical ability for indicating the main characteristics of a treatment based on the traits can be obtained by an assumptive treatment which is known as perfect position (Figure 3), the best treatments are close to this location; but treatments of the other side are the worst. Thus N80-Zs50 followed by N40-S50, N80-S50 and N40-Zs50 were perfect treatments because they were the closest entries to the position while N0-S0 (0 nitrogen application and no sulfur usage) following to N40-S0 (40 kg ha^{-1} nitrogen and no sulfur application), were on the other side and far from this position, so they are the most undesirable treatments regarding the distinction and typical abilities. The perfect treatments can be regarded as ideotypes that showed high performance of most traits, so the problem appears once the associations of traits are not always significant. Thus, these issues are serious in safflower production, in which seed yield and quality characteristics like fatty acid profile or other agronomic traits are important for obtaining high yield performance. However, utilization of multivariate statistical models with visual output has more importance toward the ideotype treatment identification in safflower. Finally, the best treatment was detected as N80-Zs50, so usage of high nitrogen and sulfur from the source of zinc sulfate is useful for obtaining the best results. This was in agreement with the findings of Ghafoor et al. (2021), who found that the higher absorption of nitrogen and sulfur, and higher yield performance were obtained with higher nitrogen application. The findings indicated the existence of reciprocal effects between N and S. It seems that the use of S in relatively alkaline soils of semi-arid regions can increase nitrogen absorption and fertilizer use efficiency by adjusting pH and optimizing rhizosphere soil conditions (Baljani et al., 2015).

The discriminating and representativeness abilities among different traits in terms of the effectiveness of fertilizer treatments largely depend on their standard deviation and the high ability is the close distance to the position of the perfect trait (Figure 4), so the most favorable traits are close to this place while traits of the other side are not the best. Thus, it can be grasped that STE, OIL, and LINN had shown high discriminating and representativeness abilities. However, this ability for all of the other remaining traits except ASH was higher than the mean of the discriminating ability. So they were significantly affected by the applied treatments (Figure 4). Also, the typical ability for presenting the figurative characteristics of a trait is determined by its angle with the horizontal axis as the mean of all traits, and a tiny angle indicates the higher discriminating and representativeness abilities for the evaluated trait. Thus, the more favorable traits (STE, OIL, and LINN) had small angles with this axis and indicated more typical ability. In contrast, the other traits (CHL, ARA, and LINL) showed large angles and had relatively lower typical ability (Figure 4). Similarly, it was shown that capitula of plants followed by oil percent and harvest index had the highest discriminative ability in safflower (Ebrahimi et al., 2023) while other findings suggest that seeds of capitulum and yield performance were the traits that had more discriminating ability in safflower (Suman et al., 2023).

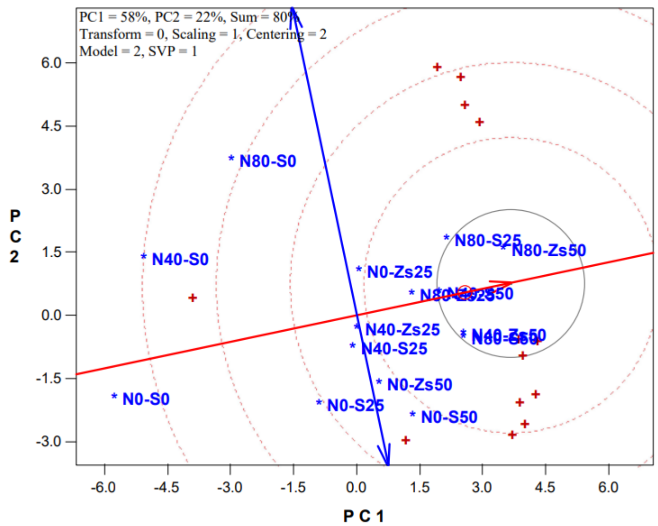

Figure 3. Ranking fertilizer treatments based on measured traits of safflower. Treatment combinations are five sulfur levels: S0 (no usage), S25 (25 kg ha^{-1} sulfur from elemental sulfur), S55 (50 kg ha^{-1} sulfur from elemental sulfur), Zs25 (25 kg ha^{-1} sulfur from zinc sulfate), Zs50 (50 kg ha^{-1} zinc sulfate), and three nitrogen levels as N0 (0 kg ha^{-1}), N40 (40 kg ha^{-1}) and N80 (80 kg ha^{-1}). Traits are: PAL, palmitic acid 16:0; STE, stearic acid 18:0; OLE, oleic acid 18:1; LINL, linoleic acid 18:2; ARA, arachidic acid 20:0; LINN linonenic acid 18:3; OIL, oil percent; ash percent (ASH); PH, plant height; SY, seed yield (SY); TSW, thousand seeds weight; and CHL, chlorophyll content.

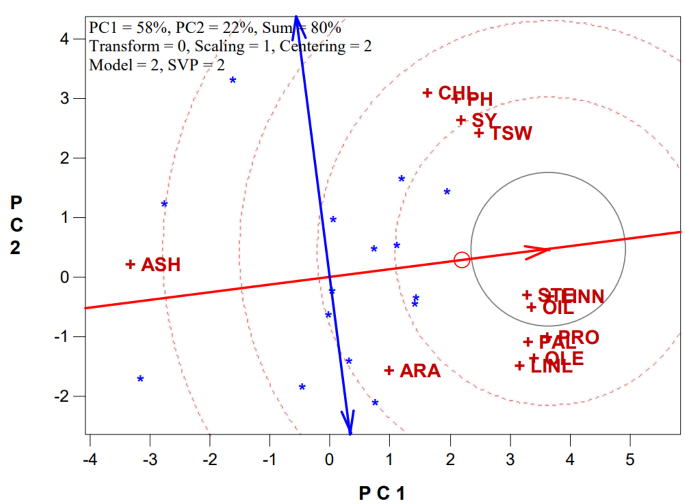

Figure 4. Ranking of traits according to discriminating and representativeness abilities. Traits are: PAL, palmitic acid 16:0; STE, stearic acid 18:0; OLE, oleic acid 18:1; LINL, linoleic acid 18:2; ARA, arachidic acid 20:0; LINN linonenic acid 18:3; OIL, oil percent; ash percent (ASH); PH, plant height; SY, seed yield (SY); TSW, thousand seeds weight; and CHL, chlorophyll content.

The effects of fertilizer treatments in terms of yield performance are shown in Figure 5, where an axis is determined as the line of yield and an arrow shows its direction. Therefore, N80-S25 (application of 80 kg ha^{-1} nitrogen and 25 kg ha^{-1} sulfur from elemental sulfur) and N80-Zs50 (application of 80 kg ha^{-1} nitrogen and 50 kg ha^{-1} sulfur from zinc sulfate sulfur) were the most favorable fertilizer treatments regarding the yield performance while N0-S0 (no application of nitrogen and sulfur) were the most undesirable treatments in this character (Figure 5). The interval of treatments from the SY axis is the measure of standard deviation, so smaller intervals are more favorable; thus, treatment N80-S25 was chosen with low variation. For example, treatment N40-S0 (40 kg ha^{-1} nitrogen and no sulfur usage) had a low yield that was even lower than mean performance (blue axis) and had a larger interval from the SY axis, so it showed more variation, and was thus one of the most unfavorable treatments. The usage of nitrogen and sulfur caused seed yield increase which emphasizes the role of nutrients in increasing vascular loading and transferring more photoassimilates to major and economic sinks such as seeds. This is an essential issue in the management of safflower especially in rainfed conditions in semiarid environments (Gürsoy, 2023). For obtaining high seed yield in safflower, balancing plant nutritional components is important. Therefore, in sodium and saline-sodium soils of semi-arid regions, the use of nitrogen without sulfur is not efficient.

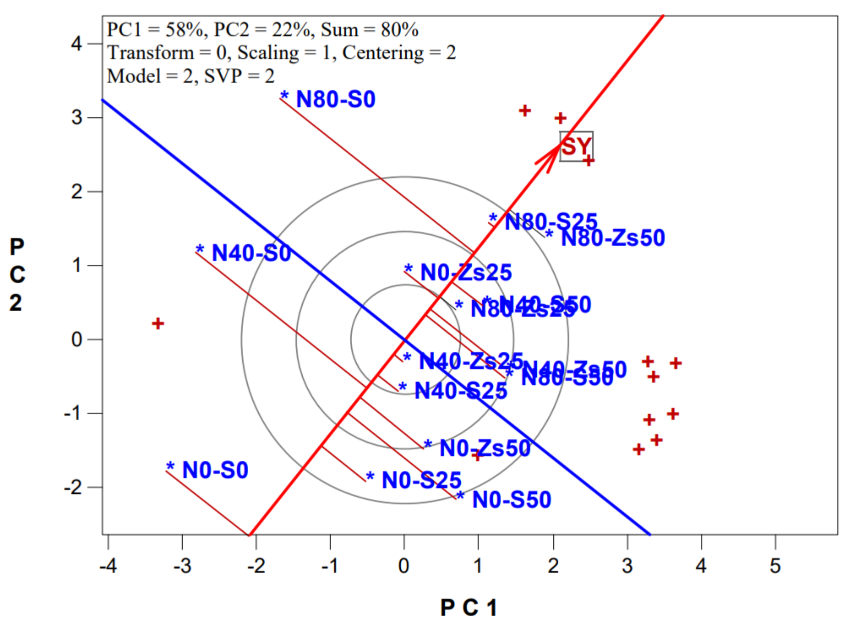

Figure 5. Biplot showing seed yield behavior against nitrogen and sulfur fertilizer levels. Treatment combinations are five sulfur levels: S0 (no usage), S25 (25 kg ha^{-1} sulfur from elemental sulfur), S55 (50 kg ha^{-1} sulfur from elemental sulfur), Zs25 (25 kg ha^{-1} sulfur from zinc sulfate), Zs50 (50 kg ha^{-1} zinc sulfate), and three nitrogen levels as N0 (0 kg ha^{-1}), N40 (40 kg ha^{-1}) and N80 (80 kg ha^{-1}). Traits are: PAL, palmitic acid 16:0; STE, stearic acid 18:0; OLE, oleic acid 18:1; LINL, linoleic acid 18:2; ARA, arachidic acid 20:0; LINN linonenic acid 18:3; OIL, oil percent; ash percent (ASH); PH, plant height; SY, seed yield (SY); TSW, thousand seeds weight; and CHL, chlorophyll content.

This investigation indicated that the fatty acid profile of safflower, consisting of saturated and unsaturated components, was influenced by nutrition management and the magnitude and source of applied fertilizers, which could affect the qualitative properties of oil. However, the soils of semiarid environments face infertility problems and nutrimental shortages, and problems include high soil pH, and the low absorbent ability of nutrients, which can influence the quality of the oil (Mekdad et al., 2022). However, the usage of 50 kg ha^{-1} elemental sulfur was essential for obtaining high quality oil with unsaturated fatty acids like linoleic, and oleic acids. Similarly, the results of other researchers emphasized the joint management of sulfur and nitrogen in rapeseed, regarding a balance between sulfur and nitrogen instead of using a single nutrient, which could result in high quality oil (Chmielewska et al., 2021).

These conclusions point to the occurrence of synergic relations among nutrients, so providing one nutrient with the optimal amount enhances the absorption of another nutrient, which increases the development and growth of crops and results in high quality and quantity of yield performance. One of the issues of interaction between sulfur and nitrogen is their influence on sulfite reductase, and nitrogen can decrease the enzymatic inhibition process (Otwell et al., 2021). Sulfite reductase had a significant role in crop growth such as in the light reaction operation of the photosynthetic system. Considering the circumstances of soils in semi-arid environments, a shortage of zinc should also be considered due to the high positive influence of zinc sulfate on seed yield and fatty acid profile. Thus, it will be interesting to investigate the interaction of zinc with sulfur and nitrogen, in the future. Finally, the results indicate that due to the synergistic effects between nitrogen and zinc, when 80 kg of nitrogen is consumed, the effect of zinc on the plant also increases. However, the source from which zinc is supplied is somewhat influential. Supplying zinc from zinc sulfate, due to the pH-reducing property of this fertilizer, affected plant growth in a better way. It seems that supplying zinc through the use of high levels of zinc sulfate improved the chemical conditions in the rhizosphere environment of safflower, and under those conditions nitrogen also showed its effect in a better way. Semi-arid regions have soil with relatively unfavorable physical and chemical conditions due to the lack of rainfall.

The results obtained in this study emphasized the existence of synergistic effects between zinc and nitrogen. The best fertilizer sources supplying zinc were zinc sulfate and elemental zinc fertilizer. Supplying the aforementioned fertilizers at high levels improved the effect of nitrogen on safflower plants and increased the developmental characteristics and yield of the plants. The results of this study underscore the significant impact of nitrogen and sulfur fertilization on the agronomic performance and fatty acid composition of safflower. Fertilizer management, particularly the application of nitrogen and sulfur, plays a crucial role in optimizing both seed yield and oil quality in safflower, especially in semi-arid regions with challenging soil conditions. The N80-Zs50 treatment (80 kg ha^{-1} nitrogen and 50 kg ha^{-1} sulfur from zinc sulfate) emerged as the most effective treatment, enhancing key agronomic traits such as plant height, seed yield, and thousand seed weight, while also promoting higher concentrations of desirable fatty acids like linolenic acid. Interestingly, the N0-S50 treatment, which involved no nitrogen application, but 50 kg ha^{-1} sulfur from elemental sulfur improved the levels of unsaturated fatty acids such as oleic, linoleic, and arachidic acids. This finding highlights the synergistic relationship between nitrogen and sulfur, where balanced nutrient application enhances not only plant growth but also the nutritional profile of safflower oil. The study suggests that a strategic combination of nitrogen and sulfur, particularly from sources like zinc sulfate, can significantly improve safflower yield and oil quality, offering valuable insights for optimizing fertilization practices in semi-arid agricultural systems.

4. Conclusions

The investigation in this study indicated the quantity and quality of the safflower agronomic traits and fatty acid composition under nitrogen and sulfur fertilizer management. The N80-Zs50 (80 kg ha^{-1} nitrogen and 50 kg ha^{-1} sulfur from zinc sulfate) was identified as the best treatment for obtaining higher values for most traits like yield and oil performance, as well as for some fatty acids like linonenic acid. Some other unsaturated fatty acids like oleic, linoleic, and arachidic acids were increased under the N0-S50 (no nitrogen application and 50 kg ha^{-1} sulfur in elemental form) treatment.

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6. Authors' Contribution

All authors have equal contributions to the implementation of the experiment, data collection, and preparation of the final draft of manuscript.

7. Conflicts of Interest

All authors declare that they have no conflicts of interest.

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References

Amirkhiz, K. F., Dehaghi, M. A., Sanavy, S. A. M. M., & Rezazadeh, A. (2021). Evaluation of changes in fatty acid profile, grain, and oil yield of *Carthamus tinctorius* L. in response to foliar application of polyamine compounds under deficit irrigation conditions. *Industrial Crops and Products,* 161, Article 113231. https://doi.org/10.1016/j.indcrop.2020.113231

Baljani, R., Shekari, F., & Sabaghnia, N. (2015). Biplot analysis of trait relations of some safflower (*Carthamus tinctorius* L.) genotypes in Iran. *Crop Research*, 50, 63-73.

Cheng, H., Yang, C., Ge, P., Liu, Y., Zafar, M. M., Hu, B., Zhang, T., Luo, Z., Lu, S., Zhou, Q., Jaleel, A., & Ren, M. (2024). Genetic diversity, clinical uses, and phytochemical and pharmacological properties of safflower (*Carthamus tinctorius* L.): an important medicinal plant. *Frontiers in Pharmacology*, 15, Article 1374680. https://doi.org/10.3389/fphar.2024.1374680

Chmielewska, A., Kozłowska, M., Rachwał, D., Wnukowski, P., Amarowicz, R., Nebesny, E., & Rosicka-Kaczmarek, J. 2021. Canola/rapeseed protein–nutritional value, functionality and food application: a review. *Critical Reviews in Food Science and Nutrition,* 61(22), 3836-3856. https://doi.org/10.1080/10408398.2020.1809342

Ebrahimi, H., Sabaghnia, N., Javanmard, A., & Abbasi, A. (2023). Genotype by trait biplot analysis of trait relations in safflower. *Agrotechniques in Industrial Crops*, 3(2), 67-73. https://doi.org/10.22126/ATIC.2023.8906.1086

Fatma, M., Masood, A., Per, T. S., Rasheed, F., & Khan, N. A. (2016). Interplay between nitric oxide and sulfur assimilation in salt tolerance in plants. *The Crop Journal,* 4(3), 153-161. https://doi.org/10.1016/j.cj.2016.01.009

Ghafoor, I., Habib-Ur-Rahman, M., Ali, M., Afzal, M., Ahmed, W., Gaiser, T., & Ghaffar, A. (2021). Slow-release nitrogen fertilizers enhance growth, yield, NUE in wheat crop and reduce nitrogen losses under an arid environment. *Environmental Science and Pollution Research International,* 28(32), 43528-43543. https://doi.org/10.1007/s11356-021-13700-4

Gürsoy, M. (2023). Morphological and biochemical changes with hormone and hydro-priming applications in safflower (*Carthamus tinctorius* L.) seedlings under salinity stress conditions. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca,* 51(3), Article 13282. https://doi.org/10.15835/nbha51313282

Janmohammadi, M., Seifi, A., Sabaghnia, N., Aghaee, A., & Dashti, S. (2017). The effect of concomitant use of nano-structured essential metals and sulfur on growth characteristics of safflower. *Annales Universitatis Mariae Curie-Sklodowska, Sectio C–Biologia,* 71(1), 41-57. https://doi.org/10.17951/c.2016.71.1.41

Kulczycki, G. (2021). The effect of elemental sulfur fertilization on plant yields and soil properties. *Advances in Agronomy*, 167, 105-181. https://doi.org/10.1016/bs.agron.2020.12.003

Manvelian, J., Weisany, W., Tahir, N. A.-R., Jabbari, H., & Diyanat, M. (2021). Physiological and biochemical response of safflower (*Carthamus tinctorius* L.) cultivars to zinc application under drought stress. *Industrial Crops and Products*, 172, Article 114069. https://doi.org/10.1016/j.indcrop.2021.114069

Mekdad, A. A., El-Sherif, A. M., Rady, M. M., & Shaaban, A. (2022). Culture management and application of humic acid in favor of *Helianthus annuus* L. oil yield and nutritional homeostasis in a dry environment. *Journal of Soil Science and Plant Nutrition,* 22(1), 71-86. https://doi.org/10.1007/s42729-021-00636-4

Ortega, J., Lopez-Hernandez, A., Garcia, H.S., & Hill, C.G. Jr. (2008). Lipase-mediated acidolysis of fully hydrogenated soybean oil with conjugated linoleic acid. *Journal of Food Science* 69(1), FEP1-FEP6. https://doi.org/10.1111/j.1365-2621.2004.tb17860.x

Otwell, A. E., Carr, A. V., Majumder, E. L. W., Ruiz, M. K., Wilpiszeski, R. L., Hoang, L. T., Webb, B., Turkarslan, S., Gibbons, S. M., Elias, D. A., Stahl, D. A., Siuzdak, G., & Baliga, N. S. (2021). Sulfur metabolites play key system-level roles in modulating denitrification. *Msystems,* 6(1), Article e01025-20. https://doi.org/10.1128/msystems.01025-20

Rahnama, A., Salehi, F., Meskarbashee, M., Khanlou, K. M., Ghorbanpour, M., & Harrison, M. T. (2024). High temperature perturbs physicochemical parameters and fatty acids composition of safflower (*Carthamus tinctorius* L.). *BMC Plant Biology,* 24(1), Article 1080. https://doi.org/10.1186/s12870-024-05781-3

Rostami, A. H., Alavi, S. M., Jabbari, H., & Jamshid, M. M. (2023). Quantitative and qualitative evaluation of safflower (Carthamus tinctorius L.) mutants in comparison with commercially released cultivars in Iran. *Agrotechniques in Industrial Crops*, 3 (1), 38-43. https://doi.org/10.22126/atic.2023.9039.1093

Sabaghnia, N., & Janmohammadi, M. (2023). Graphic analysis of compatible organic solutes treatments× trait interaction on sunflower. *Helia* 46(78), 89-99. https://doi.org/10.1515/helia-2023-0001

Sabaghnia, N., Mohebodini, M., Nikrouz-Gharamaleki, A., & Farmanpour-Kalalagh, K. (2024). Genetic diversity and morphological trait analysis of summer savory (*Satureja hortensis* L.) genotypes using GT biplot modeling. *South Western Journal of Horticulture Biology and Environment,* 15(2), 97-112.

Suman, J., Rakshit, A., Patra, A., Dutta, A., Tripathi, V. K., Mohapatra, K. K., Tiwari, R., & Krishnamoorthi, S. (2023). Enhanced efficiency N fertilizers: an effective strategy to improve use efficiency and ecological sustainability. *Journal of Soil Science and Plant Nutrition,* 23(2), 1472-1488. https://doi.org/10.1007/s42729-023-01237-z

Tian, C., Zhou, X., Liu, Q., Peng, J., Zhang, Z., Song, H., Ding, Z., Zhran, M. A., Eissa, M. A., Kheir, A. M. S., Fahmy, A. E., & Abou-Elwafa, S. F. (2020). Increasing yield, quality and profitability of winter oilseed rape (*Brassica napus*) under combinations of nutrient levels in fertiliser and planting density. *Crop and Pasture Science*, 71(12), 1010-1019. https://doi.org/10.1071/CP20328

Yan, W. (2019). LG biplot: a graphical method for mega-environment investigation using existing crop variety trial data. *Scientific Reports*, 9(1), Article 7130. https://doi.org/10.1038/s41598-019-43683-9

Zanetti, F., Angelini, L. G., Berzuini, S., Foschi, L., Clemente, C., Ferioli, F., Vecchi, A., Rossi, A., Monti, A., & Tavarini, S. (2022). Safflower (*Carthamus tinctorius* L.) a winter multipurpose oilseed crop for the Mediterranean region: Lesson learnt from on-farm trials. *Industrial Crops and Products,* 184, Article 115042. https://doi.org/10.1016/j.indcrop.2022.115042