

Enhancing safflower traits: a comparative study of fertilizer treatments on growth, yield, and oil quality

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Abstract – Efficient nutrient management plays a key role in improving safflower (*Carthamus tinctorius* L.) yield and oil quality. This study examined the effects of conventional and integrated fertilization strategies on safflower growth, yield components, oil yield, and fatty acid composition over two growing seasons (2021–2023) in Tabriz, Iran. A randomized complete block design included six treatments: an unfertilized control, 100% urea (UR), foliar NPK, and the biofertilizer PhosphoBARVAR-2 (PSB) combined with 100% UR, 50% UR, or NPK. All fertilized treatments performed significantly better than the control in most parameters across both years. The chlorophyll index increased by up to 31.12% under PSB+100% UR in Year 2 and by 38.43% under NPK in Year 1, showing improved photosynthetic activity. Seed yield rose markedly, reaching 296.18 g/m² with NPK in Year 1 and 290.78 g/m² with PSB+NPK in Year 2. Oil yield also increased sharply by 66.2% with NPK in Year 1 and 58.87% with PSB+NPK in Year 2 compared to the control. The highest oil content (37.7%) was recorded with PSB+50% UR. Integrated nutrient management (INM) treatments, *i.e.*, PSB+100% UR, PSB+50% UR, and PSB+NPK, proved highly effective. They often matched or exceeded the performance of full conventional fertilization, particularly PSB+50% UR. These treatments also enhanced oil composition. PSB+100% UR and PSB+50% UR produced the highest linoleic acid (C18:2) levels (up to 73.55%), while PSB+NPK maximized the amount of oleic acid (C18:1) to 16.05% and total unsaturated fatty acids to 83.57%. Cluster analysis clearly separated INM treatments, highlighting their distinct impact. Overall, integrating PSB with urea or NPK fertilizers, even at reduced rates, offers a promising and sustainable approach to improve both safflower yield and oil quality.

Keywords: *Carthamus tinctorius* L / integrated nutrient management / biofertilizer / seed yield / oil quality / fatty acid profile

Résumé – Étude comparative de la fertilisation conventionnelle et intégrée sur la croissance, le rendement et la qualité de l'huile du carthame en Iran. Une gestion efficace des nutriments joue un rôle clé dans l'amélioration du rendement du carthame (*Carthamus tinctorius* L.) et de la qualité de son huile. Cette étude a examiné les effets de stratégies de fertilisation conventionnelles et intégrées sur la croissance du carthame, les composantes du rendement, le rendement en huile et la composition en acides gras durant deux saisons de culture (2021–2023) à Tabriz, en Iran. Un dispositif en blocs complets randomisés comprenait six traitements : un témoin non fertilisé, un apport 100 % urée (UR), un apport foliaire NPK, et le biofertilisant PhosphoBARVAR-2 (PSB) combiné avec 100 % UR, 50 % UR ou l'engrais NPK. Tous les traitements fertilisés ont montré des performances significativement supérieures au témoin pour la plupart des paramètres, durant les deux années. L'indice de chlorophylle a augmenté jusqu'à 31,12 % avec PSB+100 % UR en année 2 et de 38,43 % avec NPK en année 1, indiquant une activité photosynthétique améliorée. Le rendement en graines a nettement augmenté, atteignant 296,18 g/m² avec NPK en année 1 et 290,78 g/m² avec PSB+NPK en année 2. Le rendement en huile a également fortement progressé, de 66,2 % avec NPK en année 1 et de 58,87 % avec PSB+NPK en année 2 par rapport au témoin. La plus forte teneur en huile (37,7 %) a été enregistrée avec PSB+50 % UR. Les traitements de gestion intégrée des nutriments (INM), c'est-à-dire PSB+100 % UR, PSB+50 % UR et PSB+NPK, se sont révélés très efficaces. Ils ont souvent égalé ou dépassé les performances de la fertilisation conventionnelle complète, en particulier

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PSB+50 % UR. Ces traitements ont également amélioré la composition en acides gras. PSB+100 % UR et PSB+50 % UR ont atteint les plus hauts taux d'acide linoléique (C18:2) (jusqu'à 73,55 %), tandis que PSB+NPK a maximisé la teneur en acide oléique (C18:1) à 16,05 % et le total des acides gras insaturés à 83,57 %. L'analyse en clusters a clairement distingué les traitements INM, soulignant leur impact spécifique. Dans l'ensemble, l'intégration du PSB avec l'urée ou les engrais NPK, même à doses réduites, constitue une approche prometteuse et durable pour améliorer à la fois le rendement du carthame et la qualité de son huile.

Mots-clés : *Carthamus tinctorius* L. / gestion intégrée des nutriments / biofertilisant / rendement en graines / qualité de l'huile / profil en acides gras

Highlights

- Optimal fertilization significantly boosted safflower chlorophyll, enhancing photosynthetic capacity.
- Specific fertilization regimes markedly increased key yield components like capitula and seed number.
- Fertilizer application substantially improved both seed yield and oil content, with some treatments nearly tripling oil yield.
- Integrated fertilization notably altered safflower oil's fatty acid profile, increasing beneficial unsaturated fatty acids.

1 Introduction

Safflower (*Carthamus tinctorius* L.) is an important oilseed crop that originated in the Near East and the Mediterranean region. Its cultivation dates back to ancient times in Persia (present-day Iran), India, and Egypt (Doğan *et al.*, 2024). The crop is valued worldwide for its edible oil, which is rich in unsaturated fatty acids, particularly linoleic and oleic acids. These fatty acids play a major role in determining the nutritional value and industrial importance of safflower oil (Sajid *et al.*, 2024). In addition to oil production, safflower contributes to animal feed through its protein-rich meal and has been used for centuries in dye making and traditional medicine (Mirghani and Elmoghtaba, 2024). As the global population continues to rise, the demand for high-quality vegetable oils and environmentally responsible agricultural systems has also increased. This trend has drawn greater research attention to crops like safflower, which offer both nutritional and industrial benefits. Improving safflower productivity and oil quality requires a deep understanding of its growth behavior, yield potential, and responses to nutrient management. The main goals in safflower cultivation are to promote healthy growth, increase seed yield, and enhance oil quality. These objectives depend closely on nutrient availability and management practices and therefore call for careful agronomic evaluation (Pushpa *et al.*, 2025).

Plant nutrition is a cornerstone of crop productivity. It supports vital physiological processes that occur throughout the plant's life cycle, from early vegetative growth to reproductive development. Adequate nutrition not only sustains biomass production but also influences the synthesis of secondary metabolites such as fatty acids and other valuable oil constituents (Mahato *et al.*, 2025). Essential macronutrients such as nitrogen (N), phosphorus (P), and potassium (K) are

vital for safflower growth and yield (Alamgeer *et al.*, 2024). Traditionally, agriculture has relied heavily on synthetic fertilizers such as urea and NPK formulations to supply these essential nutrients and enhance crop productivity. Although these fertilizers can be effective in the short term, their extensive and often inefficient use has given rise to serious environmental concerns. These include measurable increases in greenhouse gas emissions, accounting for up to 2% of global N₂O emissions, nutrient runoff that leads to eutrophication in water bodies, and progressive soil degradation caused by nutrient imbalance and a reduction in organic matter content (Govil *et al.*, 2024). For instance, excessive nitrogen application has been shown to increase nitrate concentrations in groundwater beyond the World Health Organization (WHO) safety threshold of 50 mg L⁻¹, while the continuous application of phosphate fertilizers contributes to soil hardening and a decline in microbial activity. In addition, nutrient fixation can further limit the availability of essential elements, particularly phosphorus, in many soils. This situation highlights the necessity for quantitatively assessed and sustainable nutrient management strategies that enhance nutrient-use efficiency and reduce reliance on finite synthetic inputs (Ebbisa, 2022).

Although these fertilizers may offer short-term benefits, their widespread and inefficient application results in a multitude of environmental problems, such as the rise in greenhouse gas emissions, accounting for as much as 2% of global N₂O emissions due to increased nitrification, nutrient runoff that causes eutrophication in waterways, and soil degradation resulting from nutrient imbalance and the depletion of organic matter (Govil *et al.*, 2024). For example, the overuse of nitrogen fertilizers can elevate nitrate levels in groundwater above the WHO safe drinking limit of 50 mg L⁻¹, while the continuous use of phosphate fertilizers has been associated with soil compaction and reduced microbial activity. Furthermore, nutrient fixation may restrict nutrient availability, particularly phosphorus, in most soil types. These circumstances demonstrate the urgent need for quantitatively evaluated, sustainable nutrient management strategies that can improve nutrient-use efficiency and minimize dependence on finite synthetic inputs (Ebbisa, 2022).

Integrated Nutrient Management (INM) strategies are gaining attention as sustainable approaches to address nutrient-related challenges in modern agriculture. These strategies combine chemical fertilizers with organic and biological sources to achieve balanced nutrition and improved soil health (Singh, 2020). Biofertilizers, which contain beneficial microorganisms such as phosphate-solubilizing bacteria, offer an environmentally friendly option for enhancing nutrient availability and uptake (Bashir *et al.*, 2024). These bacteria

convert insoluble soil phosphates into forms that plants can easily absorb, thereby improving phosphorus nutrition and promoting plant growth through several physiological and biochemical mechanisms. By increasing phosphorus solubility, phosphate-solubilizing bacteria application can also reduce the dependence on synthetic phosphate fertilizers (Idress *et al.*, 2025). In addition to soil-based fertilization, foliar nutrient application has proven to be an effective method for meeting plant nutritional demands during critical growth stages. Foliar feeding, particularly with NPK formulations, can help overcome soil-related constraints and improve nutrient absorption efficiency (Hemida *et al.*, 2023).

Despite the potential of INM and biofertilizers, more comparative studies are needed to evaluate their combined effects relative to conventional fertilization practices, especially for crops like safflower (Sekhar *et al.*, 2024). Previous research has shown that using a combination of fertilizers can increase seed yield, enhance oil content, and alter the fatty acid composition of safflower (Sharifi *et al.*, 2017; Ahmadpour Abnvi *et al.*, 2019; Boostanian and Ehsanzadeh, 2025). Understanding how different nutrient management regimes influence not only vegetative growth and seed yield but also the oil content and fatty acid composition, particularly the balance between saturated and unsaturated fats, with safflower oil being notably rich in linoleic (C18:2) and oleic (C18:1) acids, an essential aspect for developing optimized and sustainable cultivation practices that satisfy both yield demands and the quality standards required by consumers and the industry (Ashenafi *et al.*, 2025).

Despite the growing emphasis on sustainable nutrient management, rigorous research that compares and contrasts the effects of chemical fertilizers such as urea (UR), foliar nutrients like NPK, and phosphate-solubilizing biofertilizers such as PhosphoBARVAR-2 (PSB), along with integrated treatments involving biofertilizer application either as seed or soil inoculation, remains scarce in the context of overall safflower performance. This scarcity largely stems from the unavailability of multi-environment trials and the limited focus of previous studies on comprehensive agronomic traits and oil quality parameters. We hypothesized that blended applications of PSB in combination with reduced levels of UR or foliar NPK would be more effective in improving nutrient-use efficiency, productivity, and oil quality compared to the control. Accordingly, this study was designed to investigate and compare the effects of six different fertilizer treatments, encompassing a no-fertilizer control, conventional chemical applications (UR and NPK foliar), and integrated approaches involving PSB combined with either 100% UR, 50% UR, or NPK foliar application, on key growth parameters, yield components, seed oil content, and fatty acid composition of safflower. The overarching goal was to identify nutrient management strategies capable of enhancing safflower productivity and oil quality more sustainably than conventional high-input methods, thereby providing valuable insights into optimizing safflower cultivation within modern agricultural systems.

2 Materials and methods

A field experiment was carried out at the Research Farm of the Faculty of Agriculture, University of Tabriz, Iran, during

the 2021 and 2022 cropping seasons. The site is located at 47°39' E longitude, 38°07' N latitude, and 2211 m above sea level. The experiment followed a randomized complete block design (RCBD) with three replications. The physical and chemical properties of the soil at a depth of 0–30 cm are presented in Table 1 (Lax *et al.*, 1994). The study investigated six fertilizer treatments representing both biological and chemical sources: (1) control (no fertilizer), (2) 100% urea (UR), (3) foliar NPK, (4) PSB + 100% UR, (5) PSB + 50% UR, and (6) PSB + NPK. Urea was applied according to the soil test recommendations. In the 100% UR treatment, 115 kg N ha⁻¹ was supplied in the form of urea (46% N), equivalent to 250 kg ha⁻¹. The 50% UR treatment received 57.5 kg N ha⁻¹ (125 kg UR ha⁻¹).

For foliar feeding, an NPK formulation (20-20-20) was used, commercially available as 'YaraTera KRISTALON'. According to the manufacturer's instructions, a 10 g L⁻¹ solution was sprayed in the field. The biofertilizer PhosphoBARVAR-2 (PSB) contained two phosphate-solubilizing bacterial strains, *Pantoea agglomerans* (P25) and *Pseudomonas putida* (P13), which enhance phosphorus availability through the production of organic acids and phosphatase enzymes. The inoculant was applied at approximately 10⁸ CFU mL⁻¹, using 2 mL of inoculant per kilogram of seed, following standard agronomic practice in Iran (Emami *et al.*, 2021). The safflower (*Carthamus tinctorius* L.) variety used was ZYS, a spring type obtained from the Provincial Agricultural Jihad Organization. This variety originates from China and is characterized by early maturity, sparse secondary branching, few spines, and bright red flowers. Due to its short growth period and easy harvest, it is well suited to spring planting in temperate regions. A standard germination test was performed before sowing to ensure seed viability.

Land preparation involved plowing, disking, and leveling. The crop was hand-sown in 3 × 3 m plots, each containing five rows. The distance between rows was 50 cm, and seeds were spaced 5 cm apart within rows. The plots were separated by 1 m, with 1.5 m between blocks. Seeds were planted 5 cm deep. Thinning was done at the 3–4 leaf stage to establish a plant density of 40 plants per m². Based on soil test recommendations, UR was applied as a starter fertilizer during the first irrigation. PSB was applied as a seed coating, and foliar NPK was sprayed at the onset of flowering (Hossinzadeh and Pirzad, 2024; Raei *et al.*, 2025). Micronutrient fertilizers were also applied as foliar sprays alongside NPK when required. Irrigation was managed according to weather conditions and crop water demand. Weed control was performed manually twice during the growing season. Plant sampling and measurements were conducted at the end of the growth period.

2.1 Measurement of morphological traits, yield, and yield components

The chlorophyll index was measured at the flowering stage using a SPAD-502Plus chlorophyll meter. Ten readings were taken from each plant, randomly selected from three plants per plot (Aghighi Shahverdi *et al.*, 2019). Yield components, including the number of capitula per plant, number of seeds per capitulum, and 1000-seed weight, were determined from five randomly selected representative plants in each plot at the

Table 1. Physicochemical properties of the soil in the experimental field at two depths (0–30 cm and 30–60 cm).

Soil depth (cm)	EC (dS m ⁻¹)	pH (saturated paste)	TNV (%)	Organic carbon (%)	Total nitrogen (%)	Available phosphorus (ppm)	Available potassium (ppm)	Available Zn (ppm)	Available Fe (ppm)	Available Mn (ppm)	Sand (%)	Silt (%)	Clay (%)
0–30	2.71	7.79	7.00	0.74	0.07	15.8	410	0.72	2.6	3.8	64	22	14
30–60	2.49	7.96	7.75	0.62	0.06	11.1	400	0.42	2.1	2.7	66	20	14

Soil texture: Sandy loam

Fertilizer applications

- Urea: 250 kg ha⁻¹
- Iron sulfate (FeSO₄): 100 kg ha⁻¹
- Zinc sulfate (ZnSO₄): 20 kg ha⁻¹
- Manganese sulfate (MnSO₄): 15 kg ha⁻¹

physiological maturity stage. Although many studies employ larger sample sizes (*e.g.*, ten or more plants), five plants were deemed adequate to capture treatment differences under the relatively homogeneous field conditions of this experiment. Seed yield was expressed on a per-square-meter basis to reflect yield performance under field conditions.

An area of 1 m² was harvested from the center of each plot for yield determination. After threshing, the seeds were cleaned using a sieve, and the seed yield was recorded. Biological yield was determined by measuring the total dry weight of the aboveground biomass from the same harvested area. Representative samples were oven-dried at 70 °C for 48 h (excluding seeds), and the resulting dry weight was recorded as the biological yield (Fanaei *et al.*, 2024).

2.2 Measurement of oil content and fatty acid composition of seed oil

Oil extraction and analysis of fatty acid composition were conducted using a Soxhlet apparatus. Seed samples were oven-dried at 70 °C to constant weight to standardize moisture content prior to extraction. Ten grams of dried safflower seeds were placed in a Soxhlet cartridge, and approximately 300 mL of petroleum ether (boiling range: 40–60 °C) was used as the solvent. The extraction process lasted for 4–4.5 h, ensuring complete oil recovery as the solvent evaporated. The extracted oil was collected and stored at –24 °C for subsequent fatty acid (FA) analysis (Zafari *et al.*, 2020). Oil recovery efficiency (%) was calculated by equation (1):

$$\text{Oil content (\%)} = \left(\frac{\text{Weight of extracted oil (g)}}{\text{Weight of seed sample (g)}} \right) \times 100 \quad (1)$$

The fatty acid composition was determined following the transesterification of triglycerides into fatty acid methyl esters (FAMES), as described by Akbari *et al.* (2020) with minor modifications. Briefly, 0.05 g of the extracted oil was homogenized with 5 mL of 2% methanolic potassium hydroxide and refluxed in a water bath at 100 °C for 10 min. After cooling to room temperature, 2.18 mL of 2% methanolic boron trifluoride was added, and the mixture was refluxed again at 100 °C for 3 min to complete the methylation process. The mixture was cooled and then supplemented with 1.5 mL of hexane and 1 mL of saturated NaCl solution to facilitate phase separation. The clear upper layer containing FAMES was collected, dried over 0.5 g of anhydrous sodium sulfate, and centrifuged at 2500 rpm for 5 min. The supernatant was stored at –24 °C until further analysis by gas chromatography (GC).

FAMES were analyzed using a Unicam 4600 gas chromatograph (UK) equipped with a flame ionization detector (FID) and a BPX70 capillary column (30 m × 0.25 mm i.d. × 0.22 μm film thickness). Helium served as the carrier gas at a split ratio of 1:100. The injector and detector temperatures were set at 250 °C and 300 °C, respectively. The oven temperature program was as follows: an initial temperature maintenance at 160 °C for 5 min, an increase to 180 °C during 9 min, followed by a rise to 170 °C at a rate of 20 mL min⁻¹ and a final hold at that temperature for 35 min. Specific FAMES were identified by comparing their retention times with those of authentic standards, and their relative proportions were quantified based on corresponding peak areas.

Table 2. Comparison of safflower characteristic under different fertilizer levels in the first year.

Fertilizer treatments	Chlorophyll index	LAI	Plant height (cm)	Number of lateral stems	Number of capitula per plant
Control	33.33±1.84c	2.37±0.09c	56.2±2.46b	5.2±0.5b	9.37±1.11bc
100% UR	47.1±2.35ab	3.78±0.09a	66.83±4.19ab	8.17±1.28a	8.27±0.44bc
NPK	54.14±2.42a	3.31±0.08b	74.03±7.94a	7.83±0.94a	12.64±1.28a
PSB+100% UR	51.27±1.47ab	3.74±0.12a	65.93±1.14ab	6.53±0.66ab	9.9±0.44abc
PSB+50% UR	47.17±2.91ab	3.52±0.31ab	68.27±3.06ab	7.37±0.78ab	11.1±1.38ab
PSB+NPK	44.57±3.62b	3.38±0.04ab	63.47±1.23ab	7.47±0.6ab	7.8±0.76c
LSD=0.05	8.12	0.41	13.64	2.56	3.06

Fertilizer treatments	Capitulum weight per plant (g)	Number of seeds per capitulum	1000-Seed weight (g)	Biological yield (g/m ²)	Seed yield (g/m ²)	Oil content (%)	Oil yield (g/m ²)
Control	19.13±0.87b	31.67±3.92c	16.2±2.53b	272.93±12.84d	131.51±16.48d	19.93±1.13c	26.20±3.55d
100% UR	26.93±1.85a	57.93±5.58ab	28.65±0.81a	566.15±46.73ab	265.89±21.18ab	25.67±0.87ab	68.04±4.44ab
NPK	27±2.28a	48.27±3.96ab	28.03±1.13a	670.48±57.17a	296.18±19.04a	26.24±0.83ab	77.719±6.99a
PSB+100% UR	26.07±1.85a	44.24±2.18bc	29.44±0.86a	488.967±39.95bc	207.53±14.99c	27.27±1.2a	41.037±9.02cd
PSB+50% UR	27.18±1.73a	47.6±2.48ab	29.27±1.83a	442.61±29.97c	256.03±24.40abc	22±2.08bc	55.31±3.55bc
PSB+NPK	25.3±2.33ab	61.8±5.5a	26.69±2.06a	470.3.9±48.98bc	238.73±22.47bc	24.67±2.26ab	58.58±5.98b
LSD=0.05	6.26	14.28	5.23	120.53	53.88	4.68	14.34

UR (Urea); PSB (PhosphoBARVAR-2).

2.3 Statistical analysis

Following data collection, normality was assessed, and analysis of variance (ANOVA) was performed separately for each year using SAS software (version 9.4). Due to significant year effects, as confirmed by Bartlett's and Levene's tests, data from 2021 and 2022 were analyzed independently. Mean comparisons were carried out using the Least Significant Difference (LSD) test at a 5% probability level. Correlation analysis, path analysis, and cluster analysis were performed using Microsoft Excel 2018 and Minitab version 18 (Sarfraz *et al.*, 2024).

3 Results

3.1 Yield components and oil content

The results of the data analysis for growth traits, yield, and oil content during the first and second years of the experiment are summarized in Tables 2 and 3, respectively. The findings reveal that different fertilizer application treatments caused statistically significant variations in most measured traits of safflower plants. The chlorophyll index exhibited pronounced differences among treatments. In the first year, the foliar NPK treatment recorded the highest value (54.14), representing a 38.43% increase over the control. In the second year, the PSB + 100% UR treatment achieved the maximum chlorophyll content (50.67), corresponding to a 31.12% increase compared to the control. Fertilizer treatments significantly influenced key growth parameters such as leaf area index (LAI), plant height, and number of lateral stems in both years, indicating their combined effect on vegetative vigor and canopy development.

The highest LAI values (3.78 and 3.74) in the first year were observed under 100% UR and PSB + 100% UR treatments, respectively, approximately 37.30% and 36.63% greater than the control. These treatments also enhanced plant height and lateral branching; for example, NPK produced the tallest plants (74.03 cm) compared with the control (56.2 cm), while 100% UR and NPK resulted in 8.17 and 8.37 lateral stems per plant, respectively. In the second year, 100% UR, PSB + 100% UR, and PSB + 50% UR maintained superior performance across all traits, with LAI values ranging from 2.40 to 2.25, plant height increases of 20–25%, and the highest number of lateral stems (8.67) observed in the PSB + 50% UR treatment (Tab. 3).

Reproductive and yield-related traits also showed consistent positive responses to fertilization. The number of capitula per plant increased by 25.8% compared to the control in the first year (12.64 vs. 9.37), a trend that persisted in the second year, with the NPK treatment producing the highest values. Capitulum weight significantly improved under 100% UR, NPK, PSB + 100% UR, and PSB + 50% UR treatments (26.93–27.10 g) compared to the control in the first year. However, PSB + 50% UR achieved the highest capitulum weight in the second year, marking a 39.33% increase compared to the control.

Similarly, the number of seeds per capitulum increased by more than 48.75% under PSB + NPK in the first year, with values remaining high in the second year (36.7 seeds per capitulum) under PSB + 100% UR and PSB + 50% UR. The 1000-seed weight also showed marked improvement, reaching 29.44 g under PSB + 100% UR in the first year (a 44.97% increase compared to the control) and 29.28 g under 100% UR in the second year (Tab. 3).

Table 3. Comparison of safflower characteristic under different fertilizer levels in second year.

Fertilizer treatments	Chlorophyll index	LAI	Plant height (cm)	Number of lateral stems	Number of capitula per plant
Control	34.9±3.25c	1.75±0.15b	56±3.17b	7.93±0.9ab	5.53±0.77cd
100% UR	48.06±1.91ab	2.4±0.16a	74.93±3.14a	5.27±0.37d	6±0.31bcd
NPK	34.34±1.99c	2.09±0.15ab	67.8±2.4ab	4.67±0.24d	8.42±0.58a
PSB+100% UR	50.67±2a	2.12±0.18ab	73.2±6.91a	7.1±0.44bc	7.33±0.44ab
PSB+50% UR	43.8±4.22b	1.81±0.03b	70.2±1.62a	8.67±0.55a	7.21±0.78abc
PSB+NPK	33.2±0.73c	2.25±0.06a	68.87±6.12ab	5.6±0.87cd	4.8±0.46d
LSD=0.05	6.78	0.40	13.51	1.54	1.72

Fertilizer treatments	Capitulum weight per plant (g)	Number of seeds per capitulum	1000-Seed weight (g)	Biological yield (g/m ²)	Seed yield (g/m ²)	Oil content (%)	Oil yield (g/m ²)
Control	17.75±1.3c	24.99±2.18b	20.8±0.44d	300.33±36.34c	163.9±12.72d	22.87±1.46c	37.69±4.54b
100% UR	24.95±2.04b	34.44±0.8a	29.82±0.87a	381.52±22.45bc	213.85±22.98cd	32.5±0.67ab	69.2±5.98a
NPK	24.81±1.61b	31.85±3.73ab	28.4±0.04abc	482.01±55.91ab	233.08±23.36bc	32.4±0.81ab	75.89±9.47a
PSB+100% UR	28.52±0.99ab	34.71±2.57a	27.01±0.93bc	564.36±37.14a	278.96±8.16ab	27.43±2.42bc	76.90±9.04a
PSB+50% UR	29.26±0.39a	35.5±1.71a	26.55±1.16c	556.67±28.64a	212.33±12.17cd	37.7±1.61a	80.33±7.45a
PSB+NPK	26.94±1.44ab	36.7±1.79a	28.89±0.7ab	499.45±38.19ab	290.78±6.19a	31.5±2.48b	91.65±7.92a
LSD=0.05	4.21	7.33	2.27	117.95	52.94	5.85	26.02

UR (Urea); PSB (PhosphoBARVAR-2).

Fertilizer treatments significantly affected both biological and seed yields. In the first year, NPK application resulted in the highest biological yield (670.48 g m⁻²), which was 59.29% greater than the control. In the second year, the PSB + 100% UR and PSB + 50% UR treatments produced biological yields of 564.36 g m⁻² and 556.67 g m⁻², respectively, exceeding the control by more than 45%. These results demonstrated enhanced vegetative and reproductive growth in treated plants relative to untreated ones. A similar trend was observed for seed yield, with NPK producing the maximum value (296.18 g m⁻²) in the first year, while PSB + NPK yielded 290.78 g m⁻² in the second year (Tab. 3).

Fertilization, as presented in Tables 2 and 3, had a significant impact on both oil yield and oil quality. In the first year of treatment, the PSB+100% UR treatment produced an oil content of 27.27%, which was 26.9% higher than the control. In the second year, the highest oil content (37.7%) was recorded in the PSB+50% UR treatment. Similarly, oil yield, which represents the combined effect of seed and oil contents, reached its maximum value under NPK fertilization during the first year (77.71 g/m²), approximately three times higher than the control. In the second year, all fertilized treatments outperformed the unfertilized one, with PSB+NPK achieving the highest yield (91.65 g/m²), nearly double that of the control.

3.2 Fatty acids profile

Analysis of the fatty acid composition of safflower oil under different fertilizer treatments revealed significant variations in both saturated and unsaturated fatty acid contents (Tab. 4, Fig. 1). Among the unsaturated fatty acids, linoleic acid (C18:2) was the predominant component across all treatments, consistent with the typical fatty acid profile of

safflower oil. The highest linoleic acid concentrations were observed in PSB+50% UR (72.2%) and PSB+100% UR (73.5%), while the control, which received no fertilization, showed the lowest level (66.5%). Oleic acid (C18:1), a monounsaturated fatty acid known for enhancing oxidative stability and prolonging oil shelf life, also increased with fertilization. Its content rose from 13.5% in the control to 16.0% in PSB+NPK. Linolenic acid (C18:3), though present in smaller amounts than linoleic acid, also showed a marked increase from 0.21% in the unfertilized control to 0.45% in PSB+NPK. Long-chain fatty acids, such as eicosenoic acid (C20:1) and eicosatrienoic acid (C20:3), responded positively to fertilization as well. Eicosenoic acid increased from 0.03% in the control to 0.17% in PSB+NPK, while eicosatrienoic acid rose from 0.19% to 0.41% under the same treatments. Although slight increases were noted in saturated fatty acids such as palmitic acid (C16:0) and stearic acid (C18:0), the total saturated fatty acid content remained relatively stable across treatments, ranging from 8.2% in the control to 9.7% in PSB+NPK. Overall, the total unsaturated fatty acid content showed a clear upward trend with fertilization, reaching a maximum of 83.5% in PSB+NPK.

3.3 Correlation analysis

The correlation results (Tab. 5) revealed several significant and meaningful relationships. The chlorophyll index exhibited a strong positive correlation with both leaf area index (LAI) and plant height, suggesting its potential use as a non-destructive indicator for assessing plant growth and photosynthetic performance. Oil content demonstrated strong positive correlations with oil yield, linoleic acid, and total unsaturated fatty acids, and a negative correlation with palmitic acid. Among individual fatty acids, linoleic acid showed a strong

Table 4. Fatty acid composition of safflower oils under different fertilizer treatments.

Treatment	Palmitic (C16:0)	Stearic (C18:0)	Palmitoleic (C16:1)	Oleic (C18:1)	Eicosenoic (C20:1)	Linolenic (C18:3)	Eicosatrienoic (C20:3)	Linoleic (C18:2)	Saturated FA	Unsaturated FA
Control	6.10±0.15	2.11±0.10	0.010±0.001	13.52±1.5	0.030±0.001	0.215±0.02	0.19±0.02	66.5±4.52	8.21±1.02	79.18±3.58
100% UR	5.85±0.10	2.45±0.12	0.035±0.005	14.52±1.45	0.052±0.005	0.295±0.03	0.25±0.03	70.25±3.85	8.30±0.92	79.90±5.75
NPK	6.28±0.12	2.69±0.11	0.061±0.008	15.82±0.99	0.044±0.005	0.375±0.02	0.26±0.04	68.0±5.75	8.97±1.18	82.58±6.05
PSB+100% UR	6.77±0.14	2.35±0.09	0.058±0.005	15.05±1.55	0.152±0.001	0.308±0.03	0.33±0.03	73.55±6.38	9.12±1.22	82.84±7.01
PSB+50% UR	6.45±0.11	2.88±0.10	0.052±0.007	15.99±1.08	0.180±0.002	0.425±0.03	0.38±0.04	72.2±7.45	9.33±0.95	83.25±3.55
PSB+NPK	6.98±0.13	2.73±0.12	0.081±0.009	16.05±1.52	0.175±0.004	0.455±0.04	0.41±0.02	70.65±3.85	9.71±2.05	83.57±5.02

UR (Urea); PSB (PhosphoBARVAR-2).

positive correlation with total unsaturated fatty acids and negative correlations with saturated fatty acids, palmitic acid, and stearic acid. Furthermore, seed yield was significantly and positively correlated with the harvest index, oil yield, biological yield, and 1000-seed weight.

3.4 Path analysis

Path coefficient analysis was carried out to determine the interrelationships among yield-related traits and to identify the most influential components affecting seed yield. Standardized path coefficients (β) were obtained through multiple regression analysis, with both direct and indirect effects estimated according to the method of Wright (1960). Each model's goodness of fit was assessed using the coefficient of determination (R^2) and an overall F-test for model adequacy. The combined path analysis (Tab. 6), conducted before and after centering the data toward the yearly mean to minimize between-year variation, indicated that biological yield, capitulum weight per plant, and 1000-seed weight exerted the greatest direct effects on seed yield. The overall model was statistically significant, with an R^2 value of 0.70.

3.5 Cluster analysis

Hierarchical clustering was performed using Ward's method, based on the similarity index derived from the Pearson correlation coefficient, to evaluate the relationships among treatments. The results showed that the treatments were distinctly separated into clusters according to their fatty acid profiles. These clusters were also characterized by differences in plant physiological and yield traits. The first cluster included the control treatment, which exhibited low values for almost all growth and yield traits, indicating nutrient limitations in the absence of fertilization. The second cluster, consisting of the 100% UR and NPK treatments, was characterized by strong vegetative growth and high yield performance. These results suggest that the availability of sufficient nutrients from chemical fertilizers significantly enhanced plant development and productivity. However, this group showed relatively moderate expression of oil quality traits, implying a trade-off between yield and fatty acid composition. The third cluster, which included PSB+100% UR, PSB+50% UR, and PSB+NPK treatments, was clearly separated from the other groups. Treatments in this cluster exhibited the highest levels of unsaturated fatty acids, along with superior chlorophyll index, leaf area index (LAI), and oil yield. From a biological standpoint, the integration of PSB with chemical fertilizers not only improved nutrient uptake efficiency but also enhanced both yield and oil quality. This pattern suggests that combining biofertilizers with chemical fertilizers may provide a sustainable approach to achieving high-quality oil production while maintaining strong agronomic performance (Tab. 7, Fig. 2).

4 Discussion

The findings from this study provide comprehensive evidence that targeted fertilizer application can substantially enhance both the quantitative and qualitative performance of safflower, a conclusion strongly supported by the observed

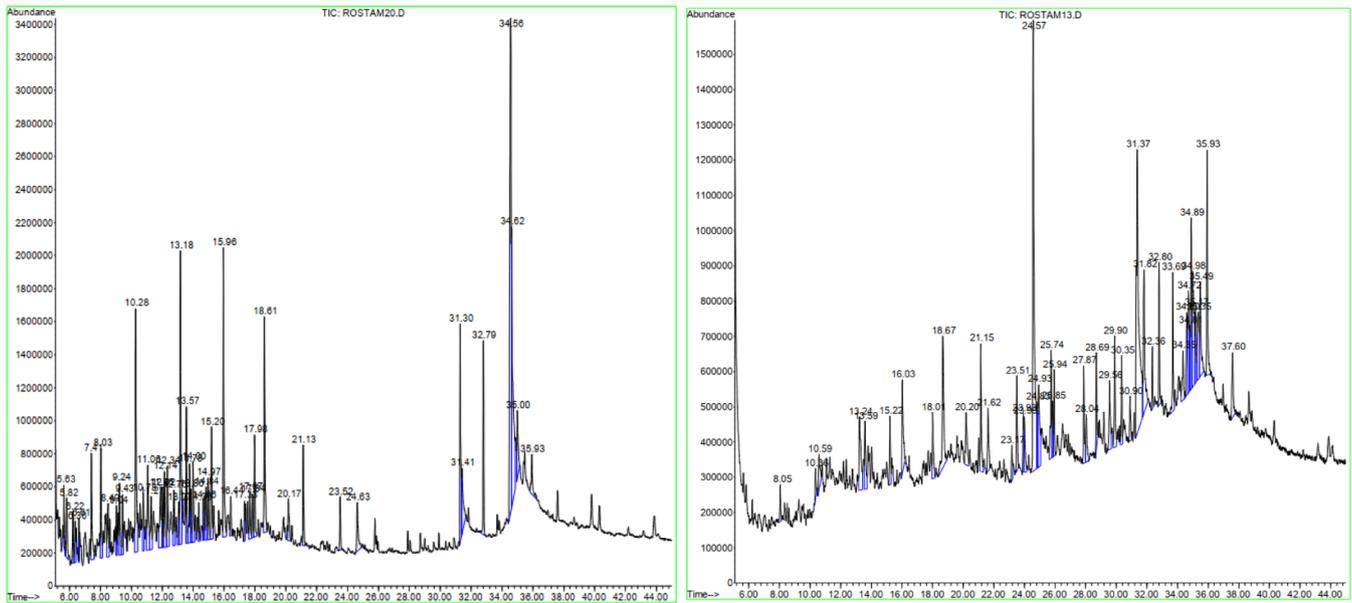


Fig 1. The GC-MS chromatogram of safflower seed oil under the influence of various fertilization treatments (right: F1, left: F6).

improvements in various growth and yield parameters discussed earlier (Zafari *et al.*, 2020). Increases in plant height, first internode length, and the number of lateral stems indicated that fertilizer treatments, particularly those involving NPK and PSB, stimulated vegetative vigor and promoted more robust canopy development. This enhanced vegetative growth, as reflected in the increased chlorophyll content and leaf area index observed in specific treatments, subsequently supported greater reproductive capacity, as evidenced by the higher number and weight of capitula, which are critical determinants of yield. The elevated number of seeds per capitulum, especially in the PSB+50% UR and PSB+NPK treatments, further underscores the significance of optimizing nutrient availability to improve reproductive efficiency and overall yield potential. This observation is consistent with the regression analysis, which identified the number of seeds per capitulum as a key predictor of seed yield.

Such gains were also evident in both economic and seed yields, indicating that proper nutrient management is imperative for increasing safflower productivity. These enhancements were associated with improved nutrient availability, higher photosynthetic efficiency, and more effective assimilate translocation into developing seeds, resulting in a general increase in biomass accumulation and yield stability across the two experimental years. The negative impact of empty capitula, as revealed in the correlation analyses, highlights the importance of maintaining an effective source–sink balance through appropriate fertilization practices to minimize resource wastage and maximize reproductive output.

A similar experiment reported that the integrated application of fertilizer sources, both chemical and biological, resulted in the highest seed yield in safflower plants, whereas the absence of fertilizer produced the lowest average yield (Mohseni Nia and Jalilian, 2012). PSB, a biofertilizer containing two strains of phosphate-solubilizing bacteria isolated from soil, enhances phosphorus availability for plant uptake. This improvement occurs through phosphatase

enzyme activity, which facilitates the secretion of organic acids and the decomposition of insoluble phosphate compounds (Gholami Kalus *et al.*, 2018). Sharifi *et al.* (2017) also demonstrated that applying PSB to safflower increased plant yield and fertility traits. Phosphate-solubilizing bacteria enhance nutrient and organic compound availability in the rhizosphere, thereby promoting plant growth and stimulating beneficial soil microbial activity. This interaction facilitates optimal water and nutrient uptake, leading to improved yields (Moradzadeh *et al.*, 2021). Similarly, the combined application of mineral nitrogen fertilizer and *Azotobacter* in sunflower significantly enhanced seed yield and its components (Arif *et al.*, 2016).

In the present study, the NPK foliar application produced the highest seed yields in the first year, while PSB+NPK treatments yielded the highest in the second year. Foliar application of NPK, as a supplementary method of plant nutrition, offers several advantages: it allows for the rapid absorption of nitrogen, phosphorus, and potassium nutrients through the leaves, thereby quickly correcting nutritional deficiencies and eliciting faster physiological responses compared to soil application (Sartika *et al.*, 2025).

NPK foliar spraying can serve as an effective method for supplying essential nutrients during critical growth stages, such as flowering and fruit set, when plants exhibit the highest nutrient demands (Phares *et al.*, 2022). This approach is particularly advantageous when root nutrient uptake is limited due to suboptimal soil pH, low temperature, or root injury. Foliar fertilization not only enhances yield and product quality but also improves nutrient-use efficiency and mitigates soil nutrient fixation processes, such as phosphorus immobilization (Niu *et al.*, 2021; Asadu *et al.*, 2024).

Beyond its effect on yield, the present study demonstrated that fertilization strategies had a profound influence on both the quantity and quality of safflower oil, extending the benefits beyond biomass accumulation. The increase in oil content under optimized treatments highlights the dual advantage of

Table 5. Simple correlation between growth, yield, and oil quality traits of safflower under different fertilizer treatments.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
1	1																						
2	0.81	1																					
3	0.86	0.88	1																				
4	0.28	0.04	0.17	1																			
5	0.05	-0.53	-0.18	0.38	1																		
6	0.79	0.82	0.91	0.42	-0.15	1																	
7	0.41	0.84	0.71	0.07	-0.74	0.76	1																
8	0.79	0.94	0.97	0.17	-0.36	0.94	0.85	1															
9	0.74	0.75	0.93	0.05	-0.09	0.89	0.65	0.91	1														
10	0.59	0.82	0.88	-0.04	-0.42	0.87	0.85	0.93	0.94	1													
11	0.05	0.29	0.48	0.26	-0.2	0.42	0.54	0.48	0.4	0.47	1												
12	0.45	0.73	0.84	0.01	-0.43	0.81	0.86	0.89	0.87	0.96	0.7	1											
13	0.05	0.17	0.1	0.07	-0.17	0.41	0.35	0.25	0.34	0.44	-0.25	0.29	1										
14	0.25	0.41	0.63	0.44	-0.12	0.76	0.67	0.67	0.69	0.73	0.78	0.84	0.36	1									
15	0.33	0.58	0.61	-0.01	-0.38	0.75	0.76	0.72	0.78	0.89	0.28	0.82	0.78	0.73	1								
16	0.33	0.48	0.66	0.27	-0.14	0.81	0.69	0.73	0.81	0.85	0.56	0.86	0.61	0.94	0.91	1							
17	0.29	0.35	0.3	0.59	-0.12	0.66	0.5	0.44	0.37	0.45	0.03	0.35	0.81	0.58	0.68	0.67	1						
18	0.15	0.41	0.52	0.31	-0.28	0.72	0.73	0.64	0.65	0.77	0.6	0.82	0.62	0.94	0.87	0.96	0.71	1					
19	0.26	0.45	0.42	0.46	-0.28	0.73	0.69	0.58	0.51	0.64	0.24	0.58	0.81	0.74	0.83	0.82	0.96	0.87	1				
20	0.76	0.68	0.6	0.55	-0.08	0.81	0.52	0.67	0.54	0.53	-0.08	0.36	0.54	0.37	0.53	0.48	0.81	0.43	0.73	1			
21	0.16	0.32	0.38	0.26	-0.18	0.66	0.58	0.51	0.58	0.67	0.2	0.61	0.89	0.75	0.91	0.89	0.86	0.9	0.94	0.57	1		
22	0.35	0.4	0.54	0.28	-0.03	0.77	0.55	0.62	0.74	0.75	0.22	0.67	0.82	0.77	0.92	0.93	0.81	0.88	0.88	0.62	0.96	1	

ns, *, and **: non-significant and significant differences at the 5% and 1%, respectively.

1. Chlorophyll index; 2. LAI; 3. Plant height; 4. Number of lateral stems; 5. Number of capitula per plant; 6. Capitulum weight per plant; 7. Number of seeds per capitulum; 8. 1000-Seed weight; 9. Biological yield; 10. Seed yield; 11. Oil content; 12. Oil yield; 13. Palmitic; 14. Stearic; 15. Palmitoleic; 16. Oleic; 17. Eicosenoic; 18. Linolenic; 19. Eicosatrienoic; 20. Linoleic; 21. Saturated FA; 22. Unsaturated FA.

Table 6. Path analysis (PAC) results for factors affecting seed yield in safflower (*Carthamus tinctorius* L.).

Characteristics	Direct ($\hat{\beta}^2$)	Indirect	Total	VIF	
Capitulum weight per plant	0.327	-0.097	0.229	1.224	
LAI	0.24	-0.025	0.215	1.509	
1000-seed weight	0.213	-0.05	0.163	2.127	
Number of lateral stems	0.493	-0.333	0.16	1.872	
Number of empty capitula per plant	0.111	0.025	0.135	1.375	
Plant height	0.164	-0.192	-0.028	2.064	
Number of seeds per capitulum	0.266	-0.386	-0.12	1.921	
First internode length	-0.216	-0.011	-0.227	1.574	
Number of capitula per plant	-0.667	0.418	-0.249	1.963	
Biological yield	-0.508	0.211	-0.297	2.201	
Pooled	N	R ₂	Adj-R ₂	F-stat	F-pvalue
	36	0.704	0.546	3.813	0.003

Table 7. Cluster analysis of traits in safflower under different fertilizer treatments.

Variable	Cluster1	Cluster2	Cluster3
Chlorophyll index	34.12	45.91	45.11
LAI	2.06	2.90	2.80
Plant height	56.10	70.90	68.32
Number of lateral stems	6.57	6.48	7.12
Number of capitula per plant	8.89	7.93	8.15
Capitulum weight per plant	18.44	25.92	27.21
Number of seeds per capitulum	28.33	43.12	43.43
1000-Seed weight	28.50	38.73	37.97
Biological yield	2866.35	5250.42	5037.44
Seed yield	1477.05	2522.54	2473.98
Oil content	25.07	29.20	27.21
Oil yield	366.22	727.75	673.05
Palmitic	6.10	6.06	6.73
Stearic	2.11	2.57	2.65
Palmitoleic	0.01	0.05	0.06
Oleic	13.52	15.17	15.70
Eicosenoic	0.03	0.05	0.17
Linolenic	0.21	0.33	0.40
Eicosatrienoic	0.19	0.26	0.37
Linoleic	66.50	69.13	72.13
Saturated FA	8.21	8.64	9.39
Unsaturated FA	79.18	81.24	83.22

enhancing economic returns while improving nutritional value, which is an important consideration for expanding the industrial and dietary applications of safflower oil (Mirdoraghi *et al.*, 2024). The marked improvement in essential fatty acids, particularly linoleic, linolenic, and oleic acids, coupled with a reduction in saturated fatty acids under the combined NPK and PSB treatments, highlights the effectiveness of integrated nutrient management in modulating lipid biosynthesis pathways. This not only elevates the health-promoting attributes of safflower oil but also enhances its marketability through a more favorable unsaturated fatty acid profile, as evidenced by the cluster analysis, which clearly distinguished treatments based on fatty acid composition. Such improvements contribute to the development of safflower oil

as a health-functional product suitable for diverse food and non-food applications. Furthermore, the threefold increase in omega-3 fatty acids observed under specific treatments highlights the potential of these integrated fertilization strategies to nutritionally enrich safflower oil and improve its overall value.

The combined application of PSB with 50% or 100% UR resulted in the highest seed oil content during the first and second years of the study. Previous research has demonstrated that applying PSB, which contains phosphate-solubilizing bacteria, can enhance seed oil content in safflower (Gholami Kalus *et al.*, 2018). These bacteria increase plant access to phosphorus by solubilizing otherwise unavailable phosphate compounds in the soil. Phosphorus plays a critical role in plant

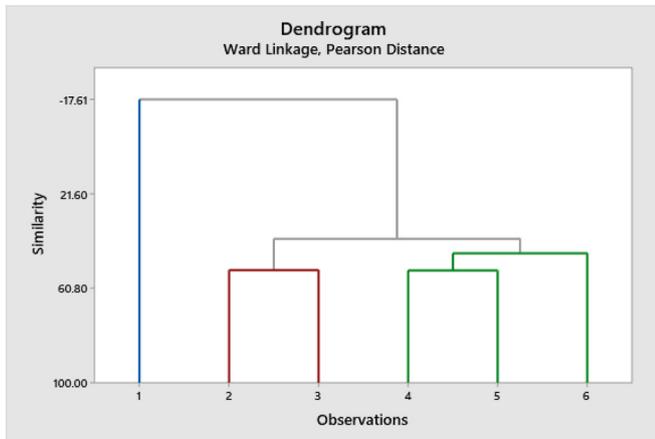


Fig. 2. Dendrogram of cluster analysis for safflower traits under different fertilizer treatments.

metabolism, particularly in energy transfer and oil biosynthesis, and improved phosphorus nutrition can therefore promote greater oil production and accumulation in seeds (Hossinzadeh and Pirzad, 2024).

Similarly, urea (UR), as a nitrogen source, also influences seed oil content. Nitrogen is a fundamental nutrient supporting both vegetative and reproductive growth, and it participates in numerous metabolic processes. An adequate nitrogen supply can enhance seed yield, thereby increasing total oil production per unit area (Zhu *et al.*, 2023). However, excessive nitrogen may reduce oil concentration in seeds even as it increases overall yield (He *et al.*, 2025), emphasizing the importance of balancing nitrogen with other essential nutrients. Thus, the combined use of PSB and UR appears to exert synergistic effects. PSB enhances phosphorus availability, potentially stimulating oil biosynthesis pathways, while urea provides the nitrogen necessary for robust vegetative growth and biomass accumulation. This synergy may explain the superior oil content and yield observed under PSB+UR treatments. Similar results were reported by Raei *et al.* (2025), who found that combining biofertilizers with urea resulted in the highest seed number, grain yield, and oil content in canola.

In the present study, treatments involving PSB+100% UR, PSB+50% UR, and PSB+NPK produced higher concentrations of the major fatty acids, *i.e.*, linoleic, palmitic, oleic, and linolenic acids. PSB was identified as a key factor influencing fatty acid composition through enhanced phosphorus bioavailability. Phosphorus is essential for the synthesis and utilization of ATP and phospholipids, which play indispensable roles in fatty acid metabolism (Nourgholipour *et al.*, 2024). The simultaneous availability of nitrogen and phosphorus likely acted synergistically to improve metabolic efficiency, which may explain the similar responses observed between PSB+50% UR and PSB+100% UR treatments (Ghaedi *et al.*, 2024). Among the tested combinations, PSB+NPK exhibited the most favorable performance, suggesting that both the nutrient source and method of application can be optimized when plant nutritional requirements are met (Chaudhary *et al.*, 2022; Hemida *et al.*, 2023). Foliar nutrient supply provides nutrients rapidly, which is particularly important during peak fatty acid synthesis stages (Phares *et al.*, 2022; Sartika *et al.*, 2025). Collectively, the

findings indicate that integrated nutrient management, while combining PSB with balanced nitrogen and potassium fertilization, enhances fatty acid content while reducing the overall reliance on chemical fertilizers (Sande *et al.*, 2024).

The path analysis revealed that biological yield, capitulum weight per plant, and 1000-seed weight exerted the strongest positive direct effects on seed yield. These traits were primarily responsible for promoting high biomass production and efficient resource allocation toward seed development. Consistent with the correlation analysis, strong positive associations were observed between seed yield and harvest index, biological yield, oil yield, and chlorophyll indices. These relationships suggest that physiological efficiency and assimilate partitioning may play a more critical role in yield formation than the mere number of reproductive units.

The strong association between chlorophyll indices and yield further underscores the importance of photosynthetic activity, which is largely governed by nitrogen availability and chlorophyll biosynthesis. This relationship was particularly evident in the NPK and PSB+100% UR treatments, where enhanced nitrogen nutrition promoted photosynthetic efficiency and, consequently, reproductive success. Interestingly, although the number of capitula per plant is generally considered a positive yield component, its indirect negative effect on seed yield in this study highlights a potential trade-off between the proliferation of reproductive organs and the efficiency of seed filling. Similar contrasting associations among yield components and oil quality traits have also been reported in safflower by Zafari *et al.* (2020).

Taken together, these findings suggest that integrating biofertilizers with chemical fertilizers offers substantial advantages for improving physiological vigor (as reflected in chlorophyll content), reproductive efficiency (through an increased number of seeds per capitulum), and ultimately oil yield. This supports the broader perspective that optimizing source–sink dynamics and fatty acid composition through targeted fertilization can enhance both the economic value and functional quality of safflower oil (Alamgeer *et al.*, 2024; Anastasi and Scavo, 2023).

Notably, this study represents one of the first systematic evaluations of integrated nutrient management (INM) using PSB in safflower cultivation under the semi-arid, cold climatic conditions of Tabriz, Iran. The results not only confirm previous reports showing that combined applications of biofertilizers and mineral nutrients (UR and NPK) exert beneficial effects but also provide direct experimental evidence of a synergistic interaction between PSB and nitrogen sources. This synergy enhanced seed yield and oil quality, particularly by improving the composition of long-chain fatty acids and photosynthetic efficiency. Collectively, these results offer new insights into nutrient management strategies aimed at achieving sustainable safflower production under semi-arid environmental conditions.

5 Conclusion

This two-year study demonstrated that fertilizer management exerted significant effects on safflower growth, yield, and oil composition. Integrated nutrient management (INM) treatments, particularly those combining PSB with urea

(UR) or foliar NPK, produced the highest biological yield, seed yield, and oil yield. These treatments also enhanced oil quality by increasing the proportion of unsaturated fatty acids, most notably linoleic and oleic acids. The results suggest that integrating PSB biofertilization with reduced urea rates can maintain high yield performance while minimizing chemical fertilizer use. Overall, the findings underscore the potential of INM as an environmentally sustainable strategy for improving both the productivity and nutritional quality of safflower under semi-arid conditions.

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Conflicts of interest

The authors declare that they have no conflict of interest or competing financial interests related to this research.

Ethical statement

Declaration of generative AI and AI-assisted technologies in the writing process:

Artificial intelligence tools were used solely for grammar, spelling, and language editing purposes. No AI tools were employed for data analysis, interpretation, or content generation in this work.

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