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Research Article

Effect of fertilizers and planting methods on safflower fatty acid profile[1]

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ABSTRACT

Soils in semi-dry areas lack sufficient nutrients for crops, so their yield depends on fertilizers and planting methods. This study aimed to investigate how the fatty acids in safflower oil change with the use of fertilizers and planting methods. Five fertilizers [F1: control; F2: 10 t ha^{-1} of farmyard manure; F3: 20 t ha^{-1} of farmyard manure; F4: NPK (130:60:45 kg ha^{-1}); F5: nano-Fe and nano-Zn] and four planting patterns (P1: 40-cm furrow planting; P2: 60-cm furrow planting; P3: 40-cm ridge planting; P4: 60-cm ridge planting) were tested. A biplot analysis explained 73 % of the variability, with the first and second principal components accounting for 54 and 19 %, respectively. The polygon-view was divided into five sections, with F3-P4 being the best for oil and protein contents, stearic (18:0), oleic (18:1), linoleic (18:2), linolenic (18:3) and lauric (12:0) acids, and iodine value. The vector-view showed a positive association among iodine value, oil content and linoleic, oleic, linolenic, stearic and lauric acids, as well as between myristic acid (14:0) and protein content, and between saponification and peroxide values. The best treatments were F4-P4, F4-P3 and F5-P4. Therefore, using NPK chemical fertilizer or nano-Fe and nano-Zn, combined with planting on a ridge with a 40 or 60-cm interrow distance, can be beneficial for producing high-quality safflower oil.

KEYWORDS: *Carthamus tinctorius* L., farmyard manure, biplot analysis.

RESUMO

Efeito de fertilizantes e tipos de plantio no perfil de ácidos graxos de cártamo

Solos em zonas semiáridas não proporcionam nutrientes suficientes para as culturas, tornando a produtividade delas dependente de fertilizantes e métodos de plantio. Objetivou-se investigar como os ácidos graxos do óleo de cártamo mudam com o uso de fertilizantes e métodos de plantio. Foram testados cinco fertilizantes [F1: controle; F2: 10 t ha^{-1} de esterco bovino; F3: 20 t ha^{-1} de esterco bovino; F4: NPK (130:60:45 kg ha^{-1}); F5: nano-Fe e nano-Zn] e quatro padrões de plantio [P1: plantio em sulco de 40 cm; P2: plantio em sulco de 60 cm; P3: plantio em canteiro de 40 cm; P4: plantio em canteiro de 60 cm]. Um tratamento biplot explicou 73 % da variabilidade, com o primeiro e o segundo componentes principais representando 54 e 19 %, respectivamente. A perspectiva poligonal foi dividida em cinco seções, sendo F3-P4 a melhor para teores de óleo e proteína, ácidos esteárico (18:0), oleico (18:1), linoleico (18:2), linolênico (18:3) e láurico (12:0) e valor de iodo. A perspectiva vetorial mostrou associação positiva entre valor de iodo, teor de óleo e ácidos linoleico, oleico, linolênico, esteárico e láurico, bem como entre ácido mirístico (14:0) e teor de proteína, e entre valor de saponificação e de peróxido. Os melhores tratamentos foram F4-P4, F4-P3 e F5-P4. Portanto, o uso de fertilizante químico NPK ou nano-Fe e nano-Zn, combinado com plantio em canteiro com distância entre linhas de 40 ou 60 cm, pode ser benéfico para a produção de óleo de cártamo de alta qualidade.

PALAVRAS-CHAVE: *Carthamus tinctorius* L., esterco bovino, análise biplot.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.), an annual crop belonging to the Asteraceae family, is one of the oldest cultivated crops. However, in recent decades, it has been underutilized and neglected by farmers (Emongor & Emongor 2023), what may be due to the scarcity of domesticated cultivars and insufficient knowledge on agronomic management (Ayalew 2020). Despite this, safflower has several appealing qualities, such as its ability to adapt to semi-arid conditions, low input requirements, strong plant vigor, resistance to abiotic stress and excellent oil quality, which have recently attracted some farmers' interest. In 2022, its total cultivated area was estimated at 0.85 million hectares, with an average seed yield of 0.8 t ha^{-1} and total production of 0.6 million tons (FAO 2022).

Safflower has well-developed and deep roots (Gholami et al. 2018). In agricultural systems,

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various factors influence the crop output and quality attributes, such as environment, agronomic techniques and genotype performance (Pasandi et al. 2018). Similarly, enhancing soil conditions and effective fertilizer management can improve quantitative and qualitative properties in safflower (Baljani et al. 2015).

Key factors affecting agronomic practices like safflower inter- and intra-row spacing include germination rate, soil fertility, water availability, production systems, cultivars and environmental conditions (Sefaoğlu & Hakan 2022). Planting arrangement significantly affects how plants access environmental elements like light and fertilizers (Fernandez et al. 2020). Plant density and planting patterns determine the rate of water and nutrient absorption, as well as the level of competition within and between species (Rehling et al. 2021). These parameters also influence crop output and yield (Zhang et al. 2021). Reducing the spacing between planting rows can lead to earlier canopy closure in the growing season, providing a higher leaf area for capturing solar radiation and producing more photosynthetic material for vegetative growth, what may result in a higher seed yield, provided that other resources are not constrained and the density increase is managed properly (Pelech et al. 2023).

For an optimal solution, it is essential to examine crop densities under various environmental and management conditions. According to Steberl et al. (2020), a greater row spacing and lower density in safflower results in more branches, capitula and higher seed weight. Conversely, reducing the row spacing in soybean increases the crop growth rates during the vegetative stage due to a greater light exposure, ultimately leading to higher yields (Vogel et al. 2021). However, very high densities can increase leaf fall due to shading, intense competition for light and reduced environmental benefits, decreasing the advantages of early and rapid growth, and reducing yield when the soil moisture and nutrients are limited (Wu et al. 2022). Species vary in their sensitivity to density, and additional agronomic practices, such as fertilizer management and planting arrangement, significantly affect the response to sowing density (Chapepa et al. 2020).

The sowing of seeds on a ridge or in a furrow can impact crop competitiveness against weeds and access to water and nutrients (Rehling et al. 2021). Al-Zubaidy & Al-Mohammad (2021) observed that safflower responds well to both in-furrow and on-ridge planting with varying nitrogen fertilizers, and that in-furrow planting with high nitrogen rates yields the best growth performance and maximum seed production.

Plants with optimal growth, particularly during weed emergence stages, and rapid seedling establishment can become more competitive (Vogel et al. 2021). Enhancing plant growth through optimal spacing and arrangement can improve yield quality by increasing the availability of fatty acids and photo-assimilates (Safdar et al. 2023).

In dry areas, agricultural productivity is mainly limited by low water availability, irregular rainfall and low organic matter in the field (Pasandi et al. 2018). These constraints underscore the importance of using farmyard manure to improve the soil physicochemical properties and explore various planting patterns. Therefore, the current study aimed to determine the impact of management techniques on safflower, in a semi-arid environment.

MATERIAL AND METHODS

The experiment was conducted at the University of Maragheh, Iran, during the 2021-2022 growing season.

The soil had a clay loam texture, with pH of 7.5, electrical conductivity of 0.8 dS m^{-1} and 0.9 g kg^{-1} of organic matter. During the growing season, temperatures ranged from 9 to 29 ºC. The experimental area (37º23′N; 46º14′E) was ploughed in the fall, using chisels. In early March, the field was prepared with ridge and furrow formations after two shallow disc harrowing operations. The trial was conducted via a factorial arrangement, in a randomized complete block design, with three replications. The first factor included five fertilizers: control (F1); 10 t ha^{-1} of farmyard manure (F2); 20 t ha^{-1} of farmyard manure (F3); NPK chemical fertilizer (130:60:45 kg ha^{-1}; F4); nano-Fe and nano-Zn micronutrients (F5). Table 1 provides some chemical characteristics of the farmyard manure. The second factor consisted of four planting patterns: planting in a furrow with a 40-cm interrow distance (P1); planting in a furrow with a 60-cm interrow distance (P2); planting on a ridge with a 40-cm interrow distance (P3); planting on a ridge with a 60-cm interrow distance (P4).

During the preparation of the ridge and furrow formations, specific volumes of well-decomposed

Table 1. Chemical characteristics of the farmyard manure used in the experiment.

P	N	K	Ca	S	OC	pH	EC	Mg	Fe	Zn	Mn
%							ds m^{-1}	%	mg ka^{-1}		
1.1	2.6	3.2	1.4	0.59	69.5	7.5	5.7	0.61	129	215	82

OC: organic carbon; EC: electrical conductivity.

farmyard manure were applied to a depth of 15 cm. The facultative safflower cultivar (Saffeh) was sown from seeds. At planting, a strip band received all recommended rates of potassium and phosphate fertilizers. One-third of the nitrogen fertilizer was applied at the sowing, stem elongation and capitulum appearance stages, with the remainder being applied in increments. Nano-Fe and Nano-Zn particles, supplied by NanoPishgaman Co. (Iran), were sprayed on the leaves at a concentration of 10 g L^{-1}, when the stem was elongating and the capitulum was beginning to appear. Throughout the growing season, water was supplied via a drip tape irrigation system. As the plants maturated, most of their leaves turned brown, and the bracts of the most recent flowering heads had a minimal green color. The standard Autonomous Communities protocol (AACC 2000) was used to determine the seed oil percentage, protein content and saponification, iodine and peroxide values. Gas chromatography, via an Agilent 6890N device from the USA, was employed to analyze the concentration of fatty acids in the samples.

The Anderson-Darling procedure was used to assess the data normality, while correlation coefficients were computed using the Pearson's coefficient. The nature of the two-way interaction data between treatment and trait (T × T) was visualized in a graph using the T × T interaction biplot model. To the biplots creation, standardized values of the trait averages were computed, and the Model-2 GGEbiplot (Yan et al. 2023) served as the basis for the analyses. It involved trait-centered data, within-trait square root of standardized variance, and no transformation.

The vector-view, ideal for visualizing interrelationships between traits and treatments, was employed based on the decomposition of trait-based singular values. Also, the polygon tool was employed according to the singular value decomposition of treatment-focused values. Plotting the symmetric scaled values of the traits and treatments resulted in T × T biplot images, where each marker represented a trait as a tester and a treatment as an entry in the image.

RESULTS AND DISCUSSION

In the T × T biplot of the mean performance of the treatments (Figure 1), a total variability of 73 % in the standardized data of the experiment was explained, with the first two principal components accounting for 54 and 19 %, respectively. In the polygon-view of the biplot, the treatments within the section defined by the lines reaching the center's origin were considered the best for each trait.

For traits such as oil percentage, protein content, stearic (18:0), oleic (18:1), linoleic (18:2),

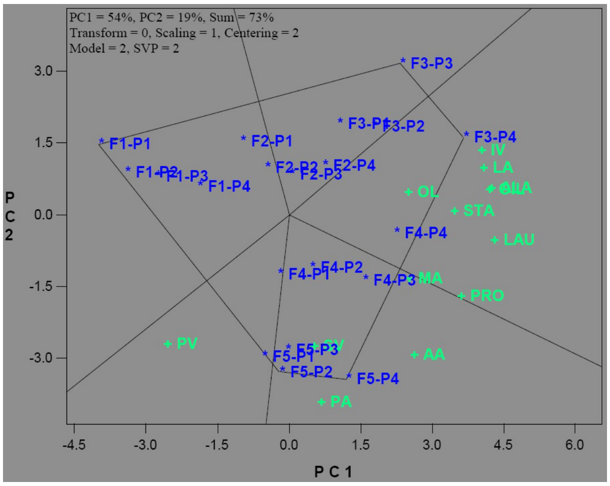

Figure 1. Polygon tool of T × T biplot for safflower fertilizer treatments. PC1: principal component 1; PC2: principal component 2; SVP: singular value partitioning. Traits: OIL: oil (%); PRO: protein (%); PA: palmitic acid (%; 16:0); STA: stearic acid (%; 18:0); OL: oleic acid (%; 18:1); LA: linoleic acid (%; 18:2); ALA: linolenic acid (18:3); AA: arachidonic acid (%; 20:4); LAU: lauric acid (12:0); MA: myristic acid (14:0); IV: iodine value; SV: saponification value; PV: peroxide value (mmol kg^{-1} of oil). Treatments: F1: control; F2: 10 t ha^{-1} of farmyard manure; F3: 20 t ha^{-1} of farmyard manure; F4: NPK chemical fertilizer (130:60:45 kg ha^{-1}); F5: nano-Fe and nano-Zn micronutrients; P1: planting in a furrow with a 40-cm interrow distance; P2: planting in a furrow with a 60-cm interrow distance; P3: planting on a ridge with a 40-cm interrow distance; P4: planting on a ridge with a 60-cm interrow distance.

linolenic (18:3) and lauric (12:0) acids, and iodine value, F3-P4 (20 t ha^{-1} of farmyard manure + planting on a ridge with a 60-cm interrow distance) emerged as the best treatment, indicating its potential as the optimal fertilizer for achieving a high oil percentage. However, while F3-P4 excelled in these traits, it did not perform as well in others, suggesting that it may not be reliable to indicate the oil percentage.

The F5-P4 (nano-Fe and nano-Zn micronutrients + planting on a ridge with a 60-cm interrow distance) was the best treatment for saponification value and fatty acids like palmitic, arachidonic and myristic (14:0) (Figure 1). Conversely, F5-P2 (nano-Fe and nano-Zn micronutrients + planting in a furrow with a 60-cm interrow distance) exhibited the best performance for peroxide value (mmol kg^{-1} of oil), indicating its suitability for achieving desirable outcomes in these traits. Additionally, F1-P1 and F3-P3 were identified as vertex treatments, with no trait detected in either sector, suggesting they are not exceptional for any of the measured traits.

It was observed that the F3-P4 treatment resulted in high oil and protein contents, along with elevated levels of stearic, oleic, linoleic, linolenic and lauric acids, and iodine value. It indicates that applying 20 t ha^{-1} of farmyard manure and planting safflower on a ridge with a 60-cm interrow distance was the most effective treatment, likely due to efficient resource utilization.

The superiority of the on-ridge planting over in-furrow planting may be attributed to the movement of the surface soil during the ridge construction, which reduced soil density, enhanced moisture penetration and increased nutrient availability. Interestingly, the application of fertilizers in the nano form led to an increase in the levels of the mentioned fatty acids, particularly short-chain fatty acids, suggesting a positive response to these treatments.

The precision of the association among traits across various treatments indicated a moderately variability. To explore relationships, a vector was drawn from the center of the graph to each trait sign. The length of the trait's vector indicates its impact on the other measured traits. The association among target traits is assessed by the cosine of angles, with sharp angles indicating a positive correlation and obtuse angles indicating a negative correlation (Yan et al. 2023).

Figure 2 illustrates positive associations among iodine value, oil percentage, linoleic, oleic, linolenic, stearic and lauric acids, as well as between myristic acid and protein content, and between saponification value and palmitic acid. These correlations suggest that a single treatment may enhance protein content and myristic acid levels. Traits such as saponification value and palmitic acid were not correlated with iodine value, oil percentage, linoleic, oleic, linolenic, stearic and lauric acids, due to nearly right angles (Figure 2). Additionally, the peroxide value was negatively correlated with the mentioned traits due to obtuse angles.

These findings align with a previous research (Campbell 2017), emphasizing the importance of providing all macronutrients and trace elements for enhancing growth and necessary processes for fatty acid synthesis. Planting on ridges and spacing out open rows may increase fatty acid levels by promoting a better leaf development and increasing the production of photo-assimilates in source-leaf tissues, facilitating their translocation to reproductive sinks (Craine & Dybzinski 2013). Additionally, the developing of root systems due to less dense soil and improved access to nutrients, water and light sources may contribute to this superiority. Thus, using farmyard manure and macronutrients, along

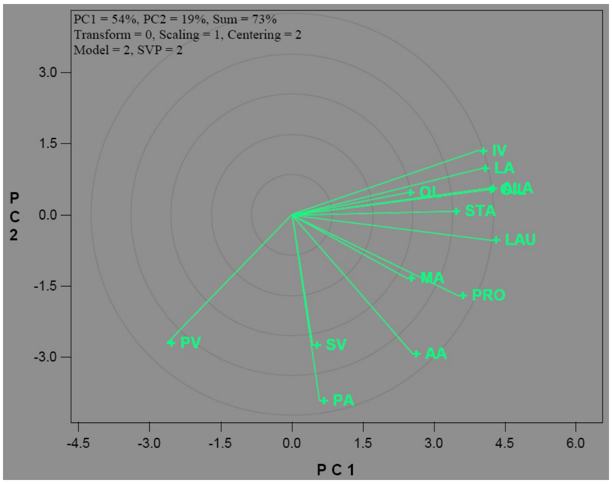

Figure 2. Vector tool of T × T biplot for safflower fertilizer treatments. PC1: principal component 1; PC2: principal component 2; SVP: singular value partitioning. Traits: OIL: oil (%); PRO: protein (%); PA: palmitic acid (%; 16:0); STA: stearic acid (%; 18:0); OL: oleic acid (%; 18:1); LA: linoleic acid (%; 18:2); ALA: linolenic acid (18:3); AA: arachidonic acid (%, 20:4); LAU: lauric acid (12:0); MA: myristic acid (14:0); IV: iodine value; SV: saponification value; PV: peroxide value (mmol kg^{-1} of oil).

with planting on ridges and maintaining long interrow spacing, increases the protein production.

In the T × T biplot analysis shown in Figure 3, an ideal treatment is characterized by incorporating multiple positive traits, which is closest to the inner circles with a sign. The perfect treatment should exhibit the highest magnitudes of the measured traits, indicated by the largest distance from the mean tester coordinate axis and the shortest length of the vector related to entries. The mean tester coordinate axis, depicted as a single-arrow line running through the biplot origin, is used as a reference for ranking treatments based on their performance. The average tester coordinate axis is divided in half by a double-arrow line, with the right-hand section indicating treatments above average and the left-hand section showing treatments below average.

According to the biplot model (Figure 3), treatments such as F4-P4, F4-P3 and F5-P4 outperformed the average, in terms of the measured traits, while treatments like F1-P2, F1-P3, F1-P4, F2-P1 and F2-P2 underperformed. Among these treatments, F4-P4, F4-P3 and F5-P4 demonstrated superiority in most traits and were closest to the location of a perfect treatment, making them the most desirable ones, in terms of the target traits. These treatments show promise for an effective safflower fertilizer management.

In Figure 4, the ideal tester is identified as the one that can effectively distinguish treatments and address the representativeness issue of the most objective trait. The center of the circles represents the ideal tester, characterized by the longest vector of traits with positive projections onto the coordinate axis of the traits' averages. A measured trait is considered ideal if it is close to the ideal tester (Baljani et al. 2015). Therefore, traits like protein content are desirable, followed by traits such as myristic, lauric and stearic acids. On the other hand, iodine value, linoleic, oleic, arachidonic and linolenic acids are moderately desirable traits. Conversely, peroxide and saponification value and palmitic acid are moderately undesirable traits, in terms of the representativeness issue of the ideal tester, and are not recommended for evaluating various treatments. Ebrahimi et al. (2023) identified the number of seeds of the main and lateral capitula as ideal traits in safflower, while the 1,000-seed weight was considered undesirable, in terms of the representativeness issue of the ideal tester.

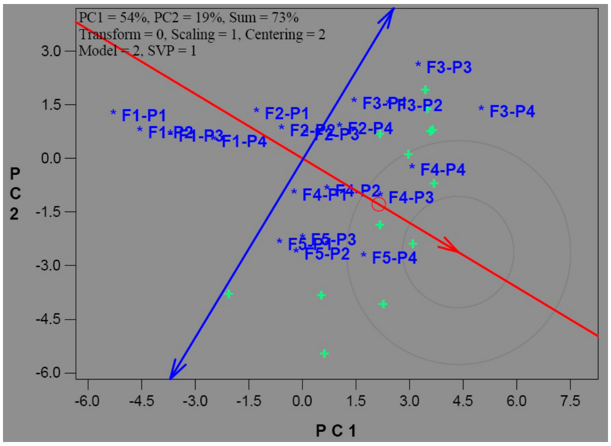

Figure 3. Perfect-treatment tool of T × T biplot for safflower fertilizer treatments. PC1: principal component 1; PC2: principal component 2; SVP: singular value partitioning. Treatments: F1: control; F2: 10 t ha^{-1} of farmyard manure; F3: 20 t ha^{-1} of farmyard manure; F4: NPK chemical fertilizer (130:60:45 kg ha^{-1}); F5: nano-Fe and nano-Zn micronutrients; P1: planting in a furrow with a 40-cm interrow distance; P2: planting in a furrow with a 60-cm interrow distance; P3: planting on a ridge with a 40-cm interrow distance; P4; planting on a ridge with a 60-cm interrow distance.

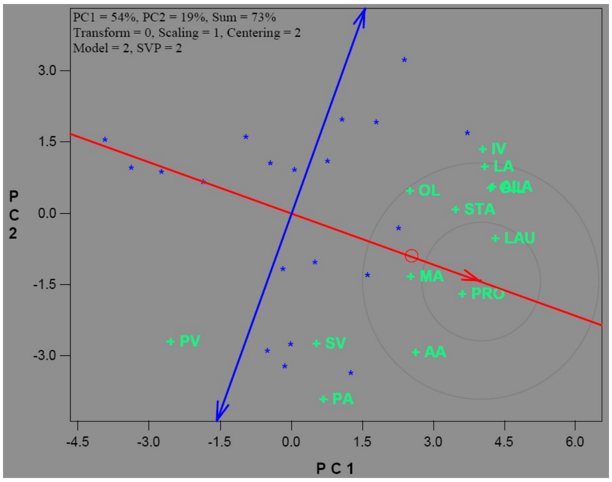

Figure 4. Perfect-trait tool of T × T biplot for safflower fertilizer treatments. PC1: principal component 1; PC2: principal component 2; SVP: singular value partitioning. Traits: OIL: oil (%); PRO: protein (%); PA: palmitic acid (%; 16:0); STA: stearic acid (%; 18:0); OL: oleic acid (%; 18:1); LA: linoleic acid (%; 18:2); ALA: linolenic acid (18:3); AA: arachidonic acid (%; 20:4); LAU: lauric acid (12:0); MA: myristic acid (14:0); IV: iodine value; SV: saponification value; PV: peroxide value (mmol kg^{-1} of oil).

In Figure 5, the performance of treatments in oil percentage as the goal trait is depicted by a moderately flat line passing through the chart origin and the chosen trait, recognized as the trait pivot, with its oval surrounding indicating a positive direction along this axis. Thus, the treatment F3-P4 (20 t ha^{-1} of farmyard manure + planting on a ridge with a 60-cm interrow distance) emerged as the best treatment for achieving a high oil content, followed by F3-P3 and F4-P4. Conversely, the treatments F1-P1, F1-P2, F1-P3 and F1-P4 exhibited the poorest performance in the oil percentage trait. Applying 20 t ha^{-1} of farmyard manure proves effective in maintaining the oil production, as it plays a crucial role in the soil function. According to Mohamed et al. (2013), the application of farmyard manure increases the oil content and stability, and the storage period.

While planting in the furrow offers certain benefits, spring sowing has significantly reduced these advantages. In the Mediterranean semi-arid region, autumn sowing can enhance soil moisture conditions due to runoff buildup and snow accumulation in the furrow. However, in the studied region, where rainfall during the growing season was limited, the results indicate that planting pattern treatments had a more significant impact when cow manure was applied. It suggests that the semi-arid region under study may have inappropriate soil characteristics, making it challenging to cultivate on ridges due to increased permeability and reduced soil density. Enhanced root development under these conditions improves plant growth, with increased photo-assimilates serving as the foundation for fatty acid synthesis.

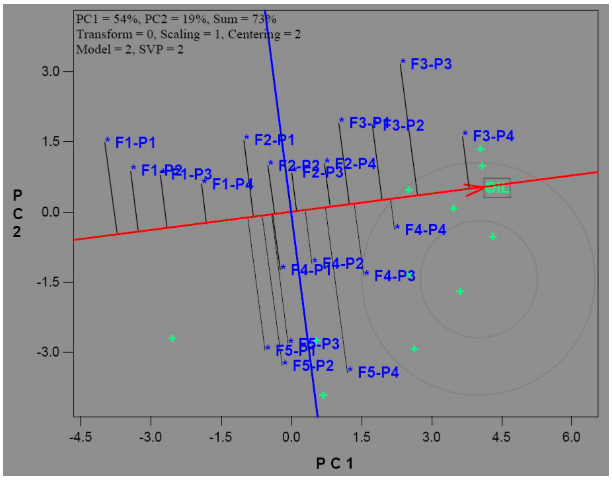

Figure 5. Ranking of treatments based on oil content (OIL) in a T × T biplot. PC1: principal component 1; PC2: principal component 2; SVP: singular value partitioning. Treatments: F1: control; F2: 10 t ha^{-1} of farmyard manure; F3: 20 t ha^{-1} of farmyard manure; F4: NPK chemical fertilizer (130:60:45 kg ha^{-1}); F5: nano-Fe and nano-Zn micronutrients; P1: planting in a furrow with a 40-cm interrow distance; P2: planting in a furrow with a 60-cm interrow distance; P3: planting on a ridge with a 40-cm interrow distance; P4: planting on a ridge with a 60-cm interrow distance.

The biplot approach offers several advantages over traditional methods, making it a valuable tool for visual data analysis. Firstly, it presents data graphically, facilitating the identification of patterns. Secondly, it simplifies pairwise comparisons among treatments or traits, enhancing interpretability. Thirdly, it enables the identification of potential positive or negative interactions between traits and treatments.

The findings of the present study reveal positive correlations among traits such as iodine value, oil percentage, linoleic, oleic, linolenic, stearic and lauric acids, as well as between myristic acid and protein content, and between saponification value and palmitic acid. It suggests that specific treatments may positively influence the protein content and myristic acid levels.

According to the perfect treatment procedure, the F4-P4, F4-P3 and F5-P4 treatments were identified as the best ones. Therefore, applying NPK chemical fertilizer or nano-Fe and nano-Zn micronutrients as fertilizers, along with planting on ridges with 40 or 60-cm interrow distances, could be beneficial for producing high-quality oil in safflower.

CONCLUSIONS

1. Farmyard manure, as well as nano-Fe and nano-Zn micronutrients, influenced the qualitative trait yield components of the safflower oil;
2. Organic fertilizers were found to enhance oil quality, when compared to conventional chemical fertilizers, while conventional chemical fertilizers rich in macronutrients notably increased the seed protein percentage;
3. It is important to prioritize the applying of farmyard manure to enhance soil physicochemical properties;
4. The use of wider row spacing and planting on ridges yielded superior results, with the best oil quality achieved with planting on a ridge with a 60-cm interrow spacing.

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