The effect of different fertilisers and planting patterns on the fatty acid profile of safflower oil

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The soils of semi-arid regions have serious nutrient deficiencies, and it seems that the qualitative aspects of crops are strongly influenced by fertiliser management and planting patterns. The current experiment was carried out to evaluate the fatty acid composition of safflower oil under the application of different fertilisers (control, farmyard manure at 10 and 20 t ha⁻¹, NPK (nitrogen, phosphorus, potassium), nano Fe+Zn) and four different planting patterns (planting in-furrow or on-ridge with inter-row distances of 40 cm or 60 cm) in the semi-arid region of north-western Iran (Maragheh). Results showed that all oil compositions significantly responded to fertiliser treatments. Utilisation of farmyard manure (FYM) increased seed oil content, and the highest oil content was recorded in planting on-ridge, which was 18% higher than the control condition. The highest percentage of seed protein was obtained by application of NPK and planting on-ridge with wider inter-row distance. Evaluation of saturated fatty acids (palmitic, stearic, myristic, lauric) showed that the application of high levels of FYM significantly increased these compositions. However, the effect of FYM20 application on main unsaturated fatty acids (linoleic acid) was much more evident, and it increased iodine value. Taken together, the results of this study indicated a positive and significant effect of the application of high levels of FYM in on-ridge planting with wider row spacing on the qualitative and nutritional aspects of safflower oil.

Keywords: inter-row distance, iodine value, oil composition, planting pattern, protein content, saturated fatty acids

INTRODUCTION

Safflower (Carthamus tinctorius) is an annual plant from the Asteraceae family. Although it is one of the oldest cultivated crops, during recent decades it has not been considered the focus of attention by farmers and has been classified as neglected and underutilised crops when compared to other crops. This may be partially due to the unavailability of domesticated cultivars and lack of sufficient information about the agronomic management of this crop (Emongor, 2010). However,
some unique characteristics of this plant, such as adaption to a wide range of environments in semi-arid regions, low input requirements, high plant vigour, abiotic stress tolerance, and high oil quality, have recently attracted the attention of farmers to some extent (Zanetti et al., 2022). During 2021, its total cultivated area was about 850,000 ha with an estimated total production of 631,000 tonnes and an average seed yield of 750 kg ha\(^{-1}\) (FAOSTAT, 2021). Although the safflower has a deep and well-expanded root, its response to nutrient management is not well known. Various factors may impact crop yield and its qualitative characteristics in agricultural systems. Safflower performance can be influenced by such factors as genotype, environment, and agronomic practices (Voronov et al., 2022; de Oliveira Neto et al., 2022). It appears that improving soil conditions and choosing efficient fertiliser management can increase the quantitative and qualitative characteristics of saffron (Pasandi et al., 2018). Agronomic practices such as inter- and intra-row spacing (plant density) of safflower vary significantly depending on environmental conditions, production systems, safflower cultivars, germination rate, soil fertility, and water availability (Steberl et al., 2020). The planting pattern and obtained plant density in the field affect the access rate of environmental factors for plants. Plant density and planting patterns will determine the rate of water and nutrient absorption, competition state within and between species that the mentioned factors determine the plant productivity and crop yield (Craine, Dybzinski, 2013). By reducing the distance between planting rows, the canopy will be closed earlier during the growing season, the field will reach the leaf area index (LAI) needed to absorb maximum solar radiation sooner, more photosynthetic material will be produced for vegetative growth and it can result in larger seed yield (Bai et al., 2016). However, the increase in density can lead to an increase in plants only up to the desired level and on the condition that other resources are not limited. Therefore, in order to choose the best options, it is necessary to evaluate the densities under different environmental and management conditions. The investigations by Steberl et al. (2020) into the response of safflower genotypes to row spacing (12 and 33 cm) and densities (40 and 75 plants m\(^{-2}\)) showed that larger spacing between rows and lower density increased the number of branches and capitula per plant and yields of florets and carthamidin. Reduction of the distance between planting rows in soybean caused an increase in the growth rate of the crop during the vegetative growth period and early reproductive growth, more light absorption throughout the growing season, and ultimately increased grain yield (Liu et al., 2010). In very high densities, the rate of leaf fall increases due to shading, severe competition for light, and deficiency of environmental factors. This may reduce the beneficial effects of early rapid growth, and with low soil moisture and nutrient deficiencies, it may cause a decrease in yield (Azari, Khajehpour, 2003).

In addition to the differences between species in terms of reaction to density, the response to sowing density is strongly influenced by other aspects of agronomic management such as fertiliser application and the planting pattern. Shahrokhnia and Sepaskha (2017) showed that safflower responded significantly to planting on-ridge or in-furrow under different nitrogen fertilisers and the best growth performance and highest seed yield was recorded for in-furrow planting with high nitrogen application (92 kg ha\(^{-1}\) of nitrogen). The position of seed planting on-ridge or in-furrow can affect the amount of accessed water, nutrients, and the competitive strength of crop plants against weeds. Crop plants can be more competitive if they have fast seedling emergence and establishment, forceful growth, particularly at stages when weeds are emerging. Improving plant growth by choosing the best planting intervals and planting patterns can improve oil quality by improving the supply of photoassimilates and fatty acids (Mohtashami, Tadayon, 2020; Pasandi et al., 2018). In semi-arid regions, low soil moisture, limited and irregular precipitation, high pH, and low soil organic matter are the major factors limiting agricultural productivity (Gan et al., 2013; Naorem et al., 2023). The mentioned limitations
explain the importance of using farmyard manures (FYM) for improving the physicochemical characteristics of soil and of investigating different planting patterns in these areas. Therefore, the purpose of the present experiment was to determine the possible effects of different management strategies/approaches (chemical and organic fertilisers, plant density, and the planting pattern; on-ridge and in-furrow sowing) on the composition of safflower oil in a semi-arid ecosystem.

MATERIALS AND METHODS

The experiment was carried out during the growing season of 2021–2022 in the research station of the Faculty of Agriculture, University of Maragheh, Iran (46°16′ E and 37°23′ N at the altitude of 1494 m). The soil textural of the research station was clay loam with pH of 7.49, electrical conductivity (EC) = 0.82 dsm⁻¹, organic matter = 0.94 g kg⁻¹, nitrogen (N) = 0.076%, phosphorus (P) = 7.61 mg kg⁻¹, and potassium (K) = 284 mg kg⁻¹. The minimum and maximum temperature of the location during the growing season was 9°C and 29°C, respectively. The amount of precipitation during the growing season was 179 mm. The land of the experimental site was ploughed by chisel during the autumn. After secondary tillage with discs in early March 2021, the ridge plough was used to split the field into ridges and furrows. The trial was carried out as a factorial (5 × 4) based on Randomized Complete Block Design with three replications. First factor was different fertiliser management including: F1 – no-fertiliser application (control), F2 – application of 10 t ha⁻¹ farmyard manure (FYM10), F3 – 20 t ha⁻¹ farmyard manure (FYM20), F4 – chemical NPK fertiliser (130 kg N ha⁻¹, 60 kg P ha⁻¹ and 45 K ha⁻¹), F5 – nanostructured Fe and Zn fertiliser. The second factor was different planting patterns and inter-row distances, including: P1 – planting in-furrow with an inter-row distance of 40 cm, P2 – planting in-furrow with an inter-row distance of 60 cm, P3 – planting on-ridge with an inter-row distance of 40 cm, P4 – planting on-ridge with an inter-row distance of 60 cm. Specified amounts of well-decomposed FYM were utilised on the surface and mixed to 15 cm depth during the preparation of the ridge and the furrow. Seeds of a facultative type of safflower ‘Saffeh’ variety were obtained from Pakan Bazr Company, Isfahan. All the recommended amounts of phosphate and potassium fertilisers were used in a strip band during planting. One-third of the amount of nitrogen fertiliser was used during planting and the rest was rationed during stem elongation (BBCH 31: 1 visibly extended internode) and capitulum appearance stages (BBCH 55: capitulum separated from youngest foliage leaves; Flemmer et al., 2015). Nano-Fe and nano-Zn particles were obtained from the Iranian Nano Pishgaman Company (Mashhad) and applied as a foliar spray (10 g L⁻¹) during stem elongation and capitulum appearance stages. Point of zero charge, the pH_pzc of nano-Zn and nano-Fe was ~6.2 and 6.7, respectively. The drip tape irrigation system was used for water supply during the growing season.

Plants were harvested at the maturity stage (BBCH: 97), most of the leaves turned a brown colour and very little green remained on the bracts of the latest flowering heads. The seed oil content was determined using the standard method AACC, 30–10 (2003), and the amount of protein was determined according to the method AACC, 4–10 (2003) (5.7 was considered as conversion factor of nitrogen to protein). The peroxide value was calculated according to AACC, 8–25 (2003). Saponification and the iodine value were determined according to AACC, 1–25 and 19–90 (2003). The iodine value is described as the amount of iodine absorbed by 100 g of oil for saturating the unsaturated bonds. For chemical and physical analysis, oil extraction was done by the cold extraction method using industrial hexane. In the first stage, the samples were mixed with hexane at a ratio of 1:5 (grain: hexane) and mixed on a magnetic stirrer at a medium speed for 24 h. To determine the amount of different fatty acids in the samples, gas chromatography methods were used. Fatty acid methyl ester was prepared according to the method reported by Ortega et al. (2004). First, 50 ml of the sample was poured into a test tube with a lid and 1 mL
of hexane was added to it. After the oil was completely dissolved, 100 µL of methoxide methanolic sodium was added to it and it was shaken for 15 min at room temperature. After the time required for the appearance of the supernatant in the tube, the hexane phase was taken out and transferred to another test tube containing some sodium sulfate (to remove excess moisture). At the time of injection into the device, 1 µL of hexane phase was used. To determine the fatty acid profile in oil samples, a gas chromatography device (Agilent 6890N, USA), equipped with an FFAP-TC capillary column 30 m long, 0.32 mm diameter of, and a thickness of the thin layer inside the tube (phase constant) of 0.25 µm was applied. The detector of the FID type device was at 250°C, and the carrier gas was nitrogen. The thermal programme used was as follows: starting the programme at a temperature of 150°C and staying at the same temperature for 1 min, then increasing the temperature to 190°C at a rate of 5°C per minute and staying at this temperature for 2 min. The methylated sample was injected into the device in a volume of 1 µL and all analysis was done on the sample. The methylated sample was injected into the device in a volume of 1 µL and all analysis was done on the sample (Ahmadzadeh et al., 2014). The gathered data related to different traits were subjected to statistical analysis in the form of an RCBD using SAS software, and if the treatment effect was significant, the LSD test was used to compare their means.

RESULTS AND DISCUSSION

Assessment of the oil content among the investigated treatments indicated that the main effects of fertiliser treatments ($P \leq 0.01$) and the planting pattern ($P \leq 0.05$) were significant on this trait (Table). The comparison of means showed that despite the application of all

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PA – palmitic acid (%), STA – stearic acid (%), OL – oleic acid (%), LAU – lauric acid (%), ALA – linolenic acid (%), MA – myristic acid (%), IV – iodine value, SV – saponification value (mg KOH/g), PV – peroxide value. For each trait, the levels of fertiliser treatments with common letters do not have statistically significant differences. F1 – no fertiliser application (control), F2 – application of 10 t ha–1 FYM, F3 – application of 20 t ha–1 FYM, F4 – chemical NPK fertiliser, F5 – Fe+Zn nano fertiliser, P1 – in-furrow planting with inter-row distance 40 cm, P2 – in-furrow planting with inter-row distance 60 cm; P3 – on-ridge planting with inter-row distance 60 cm. In each column, rows with dissimilar letters are statistically different at the 5% level.
fertilisers increasing the amount of oil significantly compared to the control, the highest amount of oil was recorded in plants grown with FYM20 (23.48%) and NPK (22.88%) (Fig. 1). On the other hand, the comparison of the means of the oil content among different densities and planting patterns showed that the highest amount of oil was recorded for on-ridge planting with an inter-row spacing of 60 cm (22.83%) and with a row spacing of 40 cm (22.24%). Overall, the application of large amounts of FYM and NPK fertiliser for the on-ridge planting method with a large inter-row distance increased the amount of oil. The effect of the planting pattern under the application of FYM10 was quite evident and on-ridge planting showed significant advantage. In this regard, Voronov et al. (2022) reported that the tillage system (mouldboard, chisel ploughing, disc ploughing) and the seeding rate significantly affected the oil content of safflower. The highest oil seed (28.75%) was recorded under deep chisel ploughing and a seeding rate of 300,000 and 400,000 seeds ha\(^{-1}\). Our findings revealed that the quantity of oil in on-ridge planting with large inter-row spacing increased and it can be due to the effective use of the available resources. It seems that due to the movement of surface soil during the preparation of the furrow and ridge construction, the reduction of soil density, higher penetration of moisture, and increased availability of required nutrients led to the advantage of on-ridge planting over in-furrow planting.

Analysis of Variance (ANOVA) of the fatty acids showed that the amount of palmitic acid was significantly affected by the use of fertilisers. Comparison of means indicated that the highest amount of palmitic acid was obtained through

![Fig. 1. The effects of different fertilisers on safflower oil content under different planting patterns. C – no fertiliser application (control), FYM10 – application of 10 t ha\(^{-1}\) FYM, FYM20 – application of 20 t ha\(^{-1}\) FYM, NPK – chemical NPK fertiliser, Fe+Zn – nano fertiliser. P1 – in-furrow planting with inter-row distance 40 cm, P2 – in-furrow planting with inter-row distance 60 cm; P3 – on-ridge planting with inter-row distance 40 cm, P4 – on-ridge planting with inter-row distance 60 cm. Statistical differences greater than LSD are statistically significant at the 5% level](image-url)
the use of inorganic macronutrient and micro-nutrient fertilisers. It is interesting to note that short-chain fatty acids significantly responded to the application of nano Fe+Zn fertilisers, and their application increased the content of the mentioned fatty acids. It seems that iron and zinc play a key role in the biosynthesis of short and saturated fatty acids. This result supports the ideas of Ghiyasi et al. (2023), who described the positive effects of foliar application of nano zinc oxide on the performance of safflower in a similar agro-climatic region. The analysis of short and saturated fatty acids such as lauric acid (12:0) indicated a significant effect of fertiliser treatments and planting patterns on the content of these fatty acids. All the examined fertilisers induced a significant increase in lauric acid content compared to the control (no-fertilisation); however, the highest content of this fatty acid was obtained through the application of high levels of farmyard manure (FYM20), which increased lauric acid content by 132% compared to the control. The use of NPK and nano-Fe+Zn could increase the lauric acid content by 107% and 96% over control, respectively. The effect of planting patterns was similar to the previously measured components, and the highest amount of lauric acid was recorded for plants grown on ridge and with large row spacing. This was also observed for myristic acid (14:0), and it seems that the response of short fatty acids to fertiliser treatments and planting patterns are partly similar: the use of FYM20, NPK, and Fe+Zn increased the amount of these fatty acids through the stimulation of related biochemical pathways.

These results are consistent with the findings of other researchers, and it seems that supplying all macro-nutrients and trace elements is necessary to improve the growth and required processes for the production of fatty acids, and the integrated management of supplying all the elements should be seriously considered (Saeidi et al., 2018; Moradzadeh et al., 2021). On the other hand, large distances between plant rows and on-ridge planting improve the amount of fatty acids through better development of the leaves. An increase in source size can enhance the production of photoassimilates and improve the translocation of photosynthetic products to the reproductive sinks and, finally, the increased availability of photoassimilates can stimulate the biosynthesis of fatty acids (Taiz et al., 2022). Furthermore, this superiority can be caused by further development of root systems due to lower soil density and better access to nutrients, water, and also the use of light sources (Shahrokhnia, Sepaskhah, 2017). The ANOVA showed that fertiliser treatments and planting patterns affected the amount of seed protein at statistical levels of 1% and 5%, respectively. The means comparison indicated that the highest protein content (23.55%) was obtained with the use of conventional macronutrient fertilisers, so that the application of NPK increased protein content by 27% compared to the control condition (Fig. 2). The application of large quantities of cow manure (FYM20) increased the amount of seed protein by about 19% compared to the control; however, no significant difference was observed between FYM20 and nano Fe+Zn. This shows that providing both groups of macro and micro-nutrients and improving the physicochemical conditions of the soil is important for improving the percentage of seed protein.

The mean comparison of protein content at different levels of the planting pattern indicated the significant superiority of on-ridge planting, and the highest amount of protein was recorded in long distances between the rows (21.56%). The lowest seed protein content was recorded for in-furrow planting with a short inter-row distance (40 cm). Altogether, on-ridge planting along with the application of FYM20 and NPK resulted in the highest seed protein content. Nitrogen is the most imperative nutrient for oil seed crops, having an active influence on all growth and seed-quality-determining processes. The seed protein content is strongly influenced by nitrogen absorption and the nitrogen sent through the process of re-transportation. It seems that a long inter-row distance, on-ridge planting, and the utilisation of FYM and NPK provided more effectively the nitrogen needed for protein production (Marschner, 2011; Taiz et al., 2022).

The content of stearic acid as a saturated long-chain fatty acid (18:0) was significantly
Fig. 2. Safflower seed protein content affected by fertiliser and different planting patterns in a semiarid region. C – no fertiliser application (control), FYM10 – application of 10 t ha⁻¹ FYM, FYM20 – application of 20 t ha⁻¹ FYM, NPK – chemical NPK fertiliser, Fe+Zn – Fe+Zn nano fertiliser. P1 – in-furrow planting with inter-row distance 40 cm, P2 – in-furrow planting with inter-row distance 60 cm, P3 – planting on-ridge with inter-row distance 40 cm, P4 – on-ridge planting with inter-row distance 60 cm

affected by fertiliser treatments ($P \leq 0.01$). Although all fertiliser applications increased the amount of this fatty acid compared to the control, the application of NPK increased the amount of this fatty acid by about 81% compared to the control. Regardless of the amount of FYM consumed, the application of these organic fertilisers increased the amount of stearic acid by 67% compared to the control. The investigation of linolenic acid as a polyunsaturated fatty acid (18:3) indicated a significant effect of fertiliser application (F) and planting patterns (P), so the interaction effects of P × F were significant for this component. Stearic acid increased strongly with the application of high levels of cow manure (FYM20) under on-ridge planting with long inter-row distance.

The use of FYM20 and on-ridge planting in open rows (60 cm) increased linolenic acid content by 97% compared to plants grown in-furrow with low row spacing (40 cm) under the of no-fertilisation condition. The significant effect of organic fertilisers and NPK on this fatty acid indicates that there was macronutrient insufficiency and micronutrient deficiency and the limitation in the soil and the significant effect of the nutrients supply on the biosynthesis of this fatty acid.

However, the application of the mentioned fertilisers requires proper soil tillage and preparation of a suitable seedbed of low density (on-ridge planting). Despite some of the described advantages of in-furrow planting, it seems that in the current experiment, the advantage of
plating in-furrow has been reduced to a great extent due to spring sowing. In autumn sowing, the moisture condition of the soil in the Mediterranean semi-arid region can be improved due to the accumulation of runoff and the trapping of snow in the furrow. Furthermore, during the studied growing season the amount of rainfall was not so high.

Investigating the qualitative components of the oil such as the iodine index (IV) also indicated a significant effect of the use of cow manure in cultivation with open rows planted on ridges. IV refers to the amount of unsaturated fatty acid in oil. In the mentioned conditions, the amount of unsaturated fatty acids increased, and this led to an increase in the iodine index. The saponification index was affected by fertiliser treatments ($P \leq 0.05$) and the use of FYM10, NPK, and Fe+Zn increased this index. Examining the amount of peroxidation indicated the decreasing effect of animal manure application on this component. This can significantly improve the storability of seeds and oil, which is very important from the point of view of post-harvest physiology.

The investigation of linoleic acid (18:2) as the main fatty acid in safflower oil was strongly influenced by the planting pattern and fertiliser management. The effect of planting patterns was evident under no fertilisation condition, so on-ridge planting increased the content of this fatty acid by 3% compared to in-furrow planting (Fig. 3). Despite the superiority of on-ridge planting, this superiority was more evident

![Fig. 3. Mean comparison of linoleic acid content extracted from safflower seeds grown under various planting pattern along with different fertiliser managements strategies. C – no fertiliser application (control), FYM10 – application of 10 t ha$^{-1}$ FYM, FYM20 – application of 20 t ha$^{-1}$ FYM, NPK – chemical NPK fertiliser, Fe+Zn – Fe+Zn nano fertiliser. P1 – in-furrow planting with inter-row distance 40 cm, P2 – in-furrow planting with inter-row distance 60 cm; P3 – on-ridge planting with inter-row distance 40 cm, P4 – on-ridge planting with inter-row distance 60 cm](image)
under the application of high levels of cow manure (FYM20). The strong response of this fatty acid to cow manure indicated the limitations in the physicochemical conditions of the soil to supply elements and factors necessary for the biosynthesis of this fatty acid. However, access to environmental sources such as light was very significant for the biosynthesis of this fatty acid. The main and interactive effects of fertiliser treatments and planting patterns were significant for the amount of lauric acid. Application of high levels of FYM20 fertiliser increased the amount of this fatty acid by 132% compared to the control (Fig. 4). The highest values of lauric acid were recorded in rows with a large distance (60 cm) and with the application of FYM (2.05%), NPK (1.83%), and Fe+Zn (1.73%). The effect of the planting patterns was more prominent under the conditions of applied farmyard manure. This indicates that due to the inappropriate soil characteristics in the studied site and semi-arid region, the use of farmyard manure and on-ridge cultivation can improve plant growth and oil quality. This improvement is caused by a reduction of soil density and the increase in permeability. The root provides factors to improve plant growth. In such conditions, the supply of more photoassimilates provides the basis for increasing the synthesis of fatty acids. The principal component analysis indicated there is a significant positive correlation between oil content, linoleic acid, linolenic acid, lauric acid,
and the iodine index (Fig. 5). Also, a significant correlation was observed between the content of seed protein and myristic acid, and the application of NPK fertilisers. Planting on-ridge had the strongest effect on the mentioned components. The lowest oil quality was related to plants grown in-furrow with no fertilisation or with the use of FYM10.

**CONCLUSIONS**

The results of the present study show that the qualitative aspects of the oil extracted from safflower seeds were affected by the fertiliser treatments. The use of organic fertilisers had a more positive effect on improving the quality of oil compared to traditional macronutrient and nano-micronutrient chemical fertilisers. However, the percentage of seed protein increased significantly with the use of traditional macronutrient chemical fertilisers. The current results stress that improving the physicochemical properties of soil in the studied region through the use of FYM as a key priority should be considered before the application of chemical fertilisers. According to the characteristics of the soils and existing limitations in dry areas, on-ridge or in-furrow planting affected some oil composition. Our result showed that on-ridge planting and the use of wide row spacing had better effects. Application of higher levels of FYM before the secondary tillage and on-ridge planting with a 60 cm inter-row distance resulted in the best oil quality.

*Fig. 5. Plot of the first two PCAs showing relation among various oil qualitative traits in safflower grown in a semi-arid region. Pro – protein content, Oil – oil content, AA – arachidonic acid, PA – palmitic acid, STA – stearic acid, OL – oleic acid, LAU – lauric acid, ALA – linolenic acid, MA: myristic acid, IV: iodine value, SV: saponification value. C: no fertiliser application (control), FYM10 – application of 10 t ha$^{-1}$ FYM, FYM20 – application of 20 t ha$^{-1}$ FYM, NPK – chemical NPK fertiliser, F5 – Fe+Zn nano fertiliser. P1 – in-furrow planting with inter-row distance 40 cm, P2: in-furrow planting with inter-row distance 60 cm; P3: on-ridge planting with inter-row distance 40 cm, P4: on-ridge planting with inter-row distance 60 cm*
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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

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