

Oil characteristics of safflower seeds under different nutrient and moisture management

Mokhtar Pasandi, Mohsen Janmohammadi[✉], Amin Abasi and Naser Sabaghnia

Department of Plant Production and Genetics, Faculty of Agriculture, University of Maragheh, P.O. Box 55181-83111, Maragheh, Iran

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Abstract

Safflower is one of the most important oilseed crops in semi-arid regions. The soil of semi-arid areas often encounters micronutrient deficiencies. However, nutrients imbalance seems to affect the quantitative and qualitative aspects of the oil as well as plant growth. Current experiment was carried out to evaluate the impact of different application practices (soil application and foliar spray) of micronutrients (Fe, Zn, Mn) on oil content, fatty acid profile and yield components of safflower under full and limited irrigations. Results showed that all of investigated traits were significantly affected by fertilizer treatment and irrigation system. The highest seed protein content was recorded for plants grown by soil application of Zn under limited irrigation condition. The highest oil content was achieved by soil application of Zn under full irrigation condition. The water deficit significantly reduced some qualitative characteristics such as oleic acid, palmitic acid, stearic acid, linoleic acid, iodine value and saponification value. The highest head number per plant, seed number per head and seed yield recorded in plants grown by soil application of Fe and Zn under full irrigation condition. Although the use of micronutrients improved qualitative characteristics in comparison with control, the best qualitative characteristics were achieved with the soil application of Zn and Fe. The elimination of micronutrient deficiencies and the balanced supply of nutrients through soil along with optimal and timely irrigation can significantly increase the efficiency of safflower production systems and improve the quality of the oil.

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Introduction

In terms of cultivated area and nutritional importance the oil crops are the second most important crops in the world after cereals. The extracted oil and by-product have an important role in maintaining the health of the community. Safflower (*Carthamus tinctorius* L.) is an annual, highly branched, herbaceous, thistle-like plant from *Asteraceae* family. Although the safflower is considered as an oilseed, it also makes an acceptable livestock forage during the vegetative

growth (Danieli *et al.* 2011). According to FAO statistics in 2016 the harvest area of safflower throughout the world was about 1,140,000 hectares and the total production was about 948,516 tones with an average yield about 832 kg ha⁻¹.

However, due to the climatic and social conditions of semi-arid regions, the main focus is on the cultivation of cereals and the utilization of cereal-fallow rotation. It seems that Safflower is best suited to be grown in rotation with cereal crops while there is not enough information about safflower crop management. All of the above

✉ Corresponding author: jmohamad@alumni.ut.ac.ir

mentioned factors have led to slow development of safflower compared to other oil crops and safflower cultivation remained a backyard crop for personal use and as a result it remained a minor and neglected crop (Emongor 2010). The use of safflower in rotation can have many benefits such as improved productivity of subsequent crops, lifting farm income, reducing the impact of disease and weeds; and producing edible and industrial quality oil and meal. Their integration offers the opportunity to enhance overall environmental, production and economic sustainability (Johnston *et al.* 2002).

Assessment of fatty acid composition in safflower seeds showed that two main unsaturated fatty acids are oleic (18:1) and linoleic acid (18:2). Oil content varies from 20 to 40 % depended on genotypes and growth conditions. They account for about 90 % of the total fatty acids and can be also responsive to environmental factors. The remaining 10 % correspond to the saturated fatty acids, palmitic (16:0) and stearic acid (18:0). Standard safflower oil contains about 6 – 8 % palmitic acid, 2 – 3 % stearic acid, 16 – 20 % oleic acid, and 71 – 75 % linoleic acid (Liu *et al.* 2016).

Climate and soil are the major factors influencing plant growth. The soil condition of the semi-arid regions is not suitable for providing the all required nutrients for optimum plant growth. The high pH and alkalinity of the soils are the major constraints on production in semi-arid region (Keshavarzi and Sarmadian 2012). The numerous other plant nutrients such as Fe, Mn, Zn and Cu also have limited bioavailability in the alkaline pH range. Although micronutrients are needed at minor amounts, their deficiencies can significantly reduce growth and yield (Marschner 2012). Micronutrients have critical roles in the photosynthesis, assimilation, and in optimizing the function of some key enzymes. For instance, Zn plays an imperative role as a co-factor of enzymes (alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase and RNA polymerase) or as a functional, structural, or regulator cofactor of a large number of enzymes. Fe involved in the manufacturing process of chlorophyll, electron carrier in respiration and photosynthesis, in the production and detoxification of radical oxygen and redox homeostasis (Hänsch and Mendel 2009). In this regard, it is worth

mentioning that finding and optimization of an efficient plant nutrition system is very important necessity in semi-arid region. However, the nutrient managements have multiple and sometimes contradictory effects on quantitative and qualitative aspects and this will make managing much more complicated. Application of micronutrient fertilizers may be carried out as soil or foliar utilization. It has been reported that in semiarid regions with low amount of precipitation foliar spray of micronutrients is a more suitable option for improving seed yield and oil characteristics compared with soil application (Galavi *et al.* 2012; Janmohammadi *et al.* 2016; Yadegari 2016). However, the efficiency of micronutrient applications methods is strongly dependent on other environmental conditions, and it is not possible to recommend a consistent method for all conditions.

On the other hand, water scarcity is one of the most important factors limiting the growth and yield of crops in semi-arid regions. Terminal water scarcity can also affect other crop management such as fertilization. Especially in the north west of Iran, which has moderately cold wet winters and hot dry summers, safflower is conservatively sown in spring (March-April), which approximately corresponds with the end of the rainy season in this region (Mirshakari *et al.* 2013). According to special agro-meteorological circumstances of semi-arid region, spring-sown safflower growth is largely restricted by terminal drought. However, some irrigation managements can partly reduce the negative effects of terminal drought stress. Limited Irrigation (LI) is a strategy to improve water use efficiency that involves the addition of limited amounts of irrigation water to essentially rain-fed crop in the last months of the spring when rainfall has dropped sharply (Reddy 2016). However, irrigation rate varies according to environmental conditions, but in the Northwest region for full irrigated field of safflower usually use for 78×10^5 liters per hectare within the dry months of growing season (May-August), while in limited irrigation only 26×10^5 liters per hectare are used as two irrigations during reproductive growth (June-July). It appears that restricted water supply during the mid-season or terminal dry spells can, however, play a critical role in enhancing and stabilizing the productivity

of spring-sown safflower. With regards to effects of environmental conditions and agronomic managements on seed yield components, oil yield and oil composition their evaluation can provide valuable information about agronomic managements. The objective of this study was to investigate the effects of soil application and foliar spray of micronutrients on the growth parameter and qualitative aspects of safflower oil in North West of Iran.

Experimental

Growth Conditions

The current experiment was carried out in Kharajou district, Maragheh, north-west of Iran (46°53' East longitude and 37°31' North latitude and at altitude of 1,780 m) during growing season 2016 – 2017 on a clay loam soil. Soil properties are shown in Table 1. The location is suitable representative of highland semi-arid zone and its climate is classified as cold semi-arid climate (Peel *et al.* 2007) with an average annual precipitation of 379 mm. This district is large elevated area and is located in the northern mountainous site of Sahand Mountain. However, most of the precipitation was recorded in winter and early spring. The metrological data of location is shown in Table 2.

Experimental Setup

Seeds of spring safflower (*Carthamus tinctorius* L.) CV. 'Esfahan' were purchased from Pakan Bazar Company. One year before the beginning of experiment were sown in isolated field in the Maragheh region and were propagated according the Sabaghnia *et al.* (2015). The experiment was laid out according Split-plot (2 × 7), with main plots arranged as an RCBD with three replications and a net plot size as 5 × 4 m. The main plot was assigned to moisture regimes including full irrigation (FI) and limited irrigation (LI). The sub-plots were allocated to fertilizer treatments which included control (no-fertilizer application), Fe²⁺_s (henceforth as Fe_s): soil application of nano-chelated iron at rate of 1.5 kg ha⁻¹, Zn²⁺_s (Zn_s): soil application of nano-

chelated zinc at rate of 1.5 kg ha⁻¹, Mn²⁺_s: soil application of nano-chelated manganese at rate of 1.5 kg ha⁻¹, Fe²⁺_f: foliar spray of nano-chelated iron at rate 1,500 ppm, Zn²⁺_f: foliar spray of nano-chelated zinc at rate 1,500 ppm, Mn²⁺_f: foliar spray of nano-chelated manganese at rate 1,500 ppm. Micronutrient fertilizers purchased from Fanavar Sepehr Parmans Co. Iran. Each plot included twenty rows, 5 m long and 75 cm apart. Seeds were sown 20 cm apart at 5 cm depth. The small terraces of 1.5 m in the interspaces was considered to prevent contamination by surface run-off containing fertilizer.

Table 1. Physical and chemical characteristics of the soil in 10-20 cm depth.

Soil property	
Clay [%]	40
Silt [%]	28
Sand [%]	22
pH	7.7
Organic carbon [g kg ⁻¹]	3.8
EC [ds m ⁻¹]	1.65
Total N [%]	0.046
P [g kg ⁻¹]	11.7
K [g kg ⁻¹]	182
Zn [g kg ⁻¹]	0.91
Fe [g kg ⁻¹]	1.26
Mn [g kg ⁻¹]	8.14

There was no incidence of pest or disease on plants during the experiment. Weeds were controlled over the growth period with hand hoeing. One mechanical weed control was performed after the fertilization by passing with rotary hoes between the rows. In foliar treatments micronutrient solution sprayed over the foliage to point of run-off during the stem elongation (BBCH=30), flowering (BBCH=30) and head growth (BBCH=71). At field capacity and at the permanent wilting point, mean soil moisture content in the top 50 cm of the soil is about 35 and 13 % by weight, respectively. Under full irrigation conditions, plots were irrigated when soil moisture content was reduced to 50 % of field capacity. Under limited irrigation plants growth were relied on rainfall and moisture stored in the soil from previous season and only two supplemental irrigations were applied during the reproductive stage (BBCH=50; head emergence and BBCH= 71; head and fruit development).

Depth of net irrigation water fraction was ~130 mm. The amount of irrigation water was calculated to restore water content in the root zone to field capacity. All other

necessary cultural practices and plant protection measures were followed uniformly for all the plots during the entire period of experimentation.

Table 2. Precipitation, mean humidity and mean temperature in crop seasons of 2016 – 2017 at evaluated site.

Parameters	March		April		May		June		July	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Pecipitation [mm]	43	62	51	49	21	18	25.9	32	13	9
Mean humidity [%]	57	63	50.2	55	40.1	51	31.7	46	30.8	34.7
Total evaporation [mm]	14	19	32	36	49	52	193	171	278	288
Mean Temperature [°C]	8.5	10.2	13.7	12.9	19.2	17.5	23.9	24.2	26.3	28

Oil Extraction and Analyses

After plant harvest at maturity stage, seeds were separated from the head and dried, then total protein was estimated by Kjeldahl method according to [Nosheen et al. \(2016\)](#). In order to perform chemical and physical analyzes, oil extraction was carried out through cold extraction method by hexane. Oil content was determined gravimetrically by extraction with technical grade hexane (Sigma-Aldrich) in a Soxhlet apparatus (Cole-Parmer, Germany) for 10 h at 40 °C. The extracted oil was stored at -18 °C for further investigation in polypropylene containers. Iodine value of oil was determined according to standard method (Cd 1-25) introduced by [AOCS \(1993\)](#). Saponification value measured according to standard method (Cd 3-25) suggested by [AOCS \(1993\)](#). Determination of fatty acids was carried out by gas chromatography apparatus (Agilent 6890N, USA). Preparation of methyl ester of fatty acids was according to [Ortega et al. \(2004\)](#). The determination of the fatty acids profile was carried out by GC equipped with a flame ionization detector and a TC-FFAP capillary column (60 m×0.25 mm internal diameter, 0.25 µm). The temperature program was as follows: starting at 150 °C and then heating to 190 °C at 5 °C/min, after 2 min followed by heating from 190 °C to 250 °C at 5 °C/min. The final temperature (250 °C) was held for 8 min. The fatty acids used as standards for the GC analyses (palmitic, stearic, and oleic acids) were from Sigma. The injector and detector temperatures were both set at 250 °C. Injections of methylated sample (1 µL) were made in the splitless mode.

Statistics

Data were analyzed by two-way variance analysis using SPSS (15.0) and comparisons among means of factors combinations were examined based on the least significance difference (LSD) test at $\alpha = 0.05$.

Results

Evaluation of plant height revealed that optimal water supply under full irrigation conditions resulted in plants higher by 31 % compared to limited irrigation conditions. The tallest plants were observed under full irrigation condition when soil was supplemented with Fe²⁺ and Zn²⁺ ([Table 3](#)). The similar statue also was observed for some seed yield parameters such as head number per plant and seed number per head. Assessment of seed yield showed that under limited irrigation it was about 24 % lower than full irrigation condition. Mean comparison among fertilizer treatments showed that soil enrichment with Fe²⁺ and Zn²⁺ under full irrigation increased the seed yield by 12 % and 14 %, over the control. The highest yield was obtained after soil enrichment with Fe²⁺ and Zn²⁺ under full irrigation. The seed yield of plants grown under full irrigation was 28 % higher than of plants grown under limited irrigation. Soil application of Fe²⁺ and Zn²⁺ increased the yield by 23 % compared with the control. In addition, regular irrigation increased the 1000-seed weight by 6 % over the limited irrigation. The heaviest seeds were recorded for plants grown in soil fertilized with Fe²⁺ and Zn²⁺ as well as in plants sprayed with Fe²⁺ ([Table 3](#)).

Analysis of variance (ANOVA) showed that seed protein content was significantly ($p < 0.01$) affected by fertilizer treatments and irrigation system (Table 3). Mean comparison between the irrigation

systems revealed that protein content of plant grown under limited irrigation was 8 % higher than at full irrigation. The highest protein content was measured in plants after soil fertilization with

Table 3. The effect of micronutrients fertilizers as soil application and foliar spraying on oil properties and yield components of safflower under different irrigation systems.

Treatments	PR	OA	IV	SV	PH	HN	SN	TSW	BY	SY
<i>Full irrigation</i>										
C	15.95 ^g	6.31 ^{cde}	170.66 ^{cde}	182.00 ^{bc}	88.00 ^b	17.33 ^{cd}	21.00 ^{cd}	28.29 ^{cde}	7,523 ^e	1,265 ^e
Fe ²⁺ _s	17.99 ^{de}	9.12 ^a	178.33 ^{ab}	196.33 ^a	102.33 ^a	23.00 ^a	25.00 ^{ab}	30.33 ^a	8,488 ^a	1,629 ^a
Fe ²⁺ _f	16.86 ^{def}	7.55 ^b	171.00 ^{cde}	181.00 ^{bc}	90.00 ^b	18.00 ^{bcd}	22.33 ^{bc}	29.64 ^{ab}	7,823 ^c	1,511 ^b
Zn ²⁺ _s	19.60 ^b	9.22 ^a	180.66 ^a	193.66 ^a	102.00 ^a	22.00 ^a	27.00 ^a	29.58 ^{ab}	8,560 ^a	1,621 ^a
Zn ²⁺ _f	17.63 ^{def}	7.29 ^{bc}	175.33 ^{bc}	173.33 ^{de}	88.66 ^b	19.66 ^b	21.00 ^{cd}	28.80 ^{bc}	7,691 ^{cd}	1,383 ^{cd}
Mn ²⁺ _s	17.86 ^{de}	6.67 ^{bcd}	174.00 ^{bc}	183.33 ^b	92.33 ^a	19.00 ^{bc}	22.33 ^{bc}	28.98 ^{bc}	8,244 ^b	1,426 ^c
Mn ²⁺ _f	17.31 ^{ef}	6.97 ^{bcd}	171.33 ^{cde}	178.00 ^{cd}	87.66 ^b	17.66 ^{bcd}	19.00 ^{cde}	28.53 ^{cd}	7,575 ^d	1,312 ^{de}
<i>Limited irrigation</i>										
C	18.20 ^{de}	5.56 ^e	156.00 ^f	167.66 ^{fg}	66.00 ^d	12.00 ^f	14.66 ^f	27.56 ^{efg}	5,603 ^g	1,008 ^h
Fe ²⁺ _s	19.13 ^{bc}	6.90 ^{bcd}	166.33 ^e	170.66 ^{efg}	70.50 ^{cd}	17.00 ^{cd}	18.00 ^{def}	28.23 ^{cdef}	6,292 ^e	1,175 ^{fg}
Fe ²⁺ _f	18.30 ^{de}	6.90 ^{bcd}	172.33 ^{cd}	174.00 ^{de}	73.80 ^c	16.00 ^{de}	15.66 ^{ef}	27.79 ^{defg}	5,909 ^f	1,134 ^{fg}
Zn ²⁺ _s	22.28 ^a	6.46 ^{bcd}	168.00 ^{de}	172.00 ^{ef}	73.50 ^c	18.00 ^{bcd}	17.66 ^{def}	27.60 ^{efg}	6,330 ^e	1,183 ^f
Zn ²⁺ _f	19.60 ^b	6.31 ^{cde}	168.00 ^{de}	171.33 ^{efg}	71.40 ^{cd}	14.66 ^e	18.00 ^{def}	27.37 ^g	6,045 ^f	1,141 ^{fg}
Mn ²⁺ _s	18.50 ^{cd}	5.88 ^{de}	159.33 ^f	169.33 ^{efg}	68.70 ^{cd}	17.00 ^{cd}	17.00 ^{ef}	28.02 ^{defg}	5,868 ^f	1,108 ^g
Mn ²⁺ _f	17.75 ^{def}	5.46 ^e	160.66 ^f	166.33 ^g	71.40 ^{cd}	14.33 ^e	16.00 ^{ef}	27.47 ^{fg}	5,907 ^f	1,157 ^{fg}
Statistical significance										
Fertilizer (F)	**	**	**	**	*	**	**	**	**	**
Irrigation (I)	**	**	**	**	**	**	**	**	**	**
F×I	*	*	**	*	NS	NS	NS	*	**	*
CV	3.24	8.93	1.83	1.72	4.79	8.06	11.12	1.66	2.14	7.53

C: control (No-fertilizer application); (s) and (f) in the subscript of the fertilizer refer to soil application and foliar feeding; PR: seed protein content [%]; OA: Oleic acid [%]; IV: iodine value of oil; SV: saponification value; PH: plant height [cm]; HN: head number per plant; SN: seed number per head; TSW: thousand seed weight; BY: biological yield [kg ha⁻¹]; SY: seed yield [kg ha⁻¹]. In each column, values with similar letter(s) are not significantly different at the 5 % level of probability. NS = Not significant; * = Significant at 5 % level of probability; ** = Significant at 1 % level of probability.

Zn²⁺ under limited irrigation, while the lowest amounts were related to lack of any fertilizer under full irrigation conditions. Evaluation of the oil content showed that the effect of irrigation and fertilizers treatment was significant. Oil content is one of rare traits that can be considered both as a quantitative and qualitative characteristic. Reducing irrigation frequencies under limited irrigation considerably reduced (by 9 %) the oil content in comparison with full irrigation. The highest oil content was recorded for plants grown in soil with addition of Zn²⁺ and followed with plant grown by foliar spray of Zn under full irrigation system (Fig. 1). Also comparison of fertilizer treatment under limited irrigation showed that the highest oil content was related to

soil application of Zn²⁺. Similar, though smaller effects, were observed for Fe²⁺. Assessment of oleic acid revealed a significant effect of irrigation system and fertilizer treatment ($p < 0.01$). Oleic acid content in limited irrigation conditions was about 19 % lower than full irrigation. The highest oleic acid content was recorded for plant grown in soil enriched by Zn²⁺ or Fe²⁺, both under full irrigation condition (Table 3). Water shortage considerably reduced iodine value of oil (by 6 %). The highest iodine value was related to plant grown in soil fertilized with Zn²⁺ and Fe²⁺ under full irrigation condition while the lowest value was recorded for plant grown with no-fertilizer, or foliar-applied Mn²⁺ under limited

irrigation (Table 3). A similar trend also was observed for saponification values (Table 3).

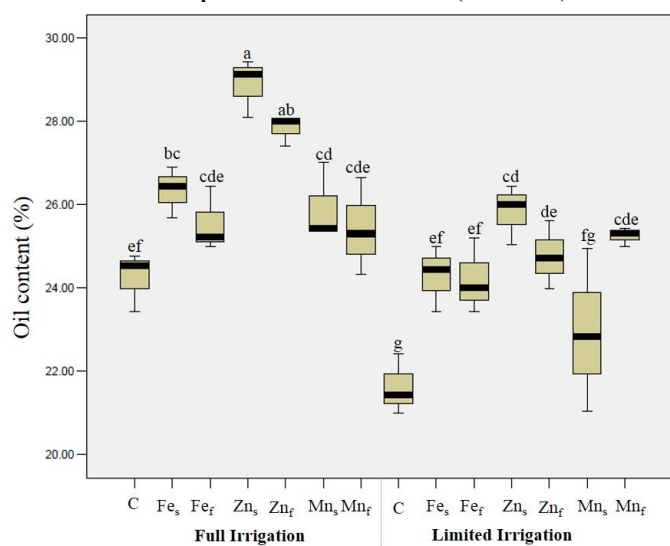


Fig. 1. Effect of fertilizers with micronutrient (Fe²⁺, Zn²⁺, Mn²⁺) applied into soil or by spraying (indexes _s and _f, respectively) on total oil content in safflower seeds under different moisture regimes. Values with different superscripts are significantly different (*p* < 0.05). All values are mean ± SEM.

The irrigation system and fertilizer treatment significantly affected the content of palmitic acid (Fig. 2). The content of this fatty acid under limited irrigation conditions was about 18 % lower than under full irrigation conditions. Effect of fertilizers with micronutrient (Fe²⁺, Zn²⁺, Mn²⁺) applied into soil or by spraying (indexes _s and _f, respectively) on total oil content in safflower seeds under different moisture regimes are shown on Fig. 1, while values with different superscripts are significantly different (*p* < 0.05). ANOVA for stearic acid (Fig. 3) and linoleic acid content (Fig. 4) revealed that the effects of irrigation system, fertilizer treatments as well as their interaction effects were significant. The content of linoleic acid in plants grown under limited irrigation was about 6% lower than under full irrigation. The highest values were recorded for plants fertilized through both soil and foliar spray with Fe²⁺ under full irrigation condition (Fig. 4). When regular water supply was applied, foliar spray of Fe²⁺ and Zn²⁺ had a better effect on linoleic acid content in comparison with other fertilization methods.

Cluster analysis of traits in response to the treatments (Fig. 5) indicated that oil content,

protein percent and palmitic acid content were impacted similarly (cluster I); the highest values of the above mentioned traits were obtained for samples of soils with applied Zn²⁺ fertilizer.

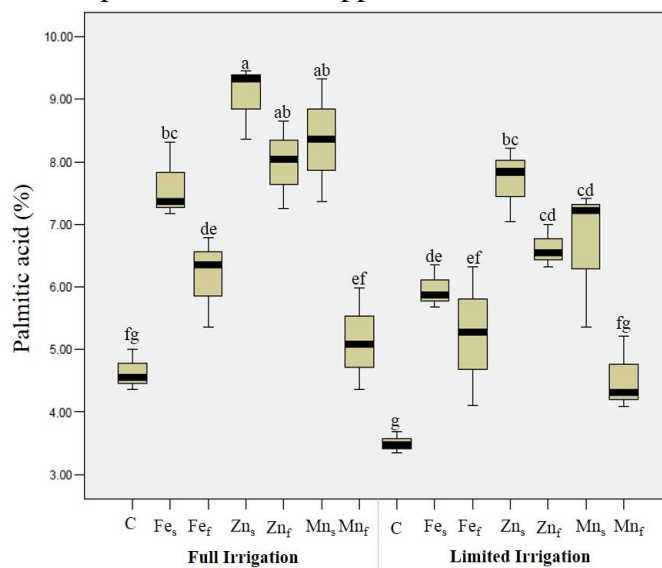


Fig. 2. Effect of fertilizers with micronutrient (Fe²⁺, Zn²⁺, Mn²⁺) applied into soil or by spraying (indexes _s and _f, respectively) on palmitic acid content in safflower seeds under different moisture regimes. Values with different superscripts are significantly different (*p* < 0.05). All values are mean ± SEM.

The second cluster groups of oleic acid, iodine value of oil, saponification value and agronomic traits such as seed yield, head number, plant height, biological yield, seed number per head. The highest values for these parameters were obtained for plants grown in soils with applied Zn²⁺ and Fe²⁺ under full irrigated condition.

The third cluster consists of traits such as content of stearic acid, linoleic acid and 1000-seed weight, which were positively affected by foliar application of Fe²⁺ and Zn²⁺ under full irrigated condition (Fig. 5). Further, cluster analysis of fertilizer treatments could classify them into three groups. The first group was control (no fertilizer applied) resulting in the poorest performance of plants. The second cluster grouped the treatments of Mn_s, Fe_f, Zn_f and Mn_f. These treatments improved the qualitative and quantitative traits in comparison with the control, however, difference among them were not significant. The third cluster includes the treatments with Fe_s and Zn_s that resulted in the highest improvements in the studied traits (Fig. 6).

Discussion

Our study confirmed that qualitative traits of seeds and oil are significantly affected by agronomic managements. Our results are consistent with results of some previous works (Baldini *et al.* 2000; Movahhedy-Dehnavy *et al.* 2009; Omidi *et al.* 2010). Although limited irrigation is done to reduce water stress and prevent loss of yield, amount and distribution of rainfall in the experimental area appear inappropriate since even limited irrigation failed to fully eliminate the impacts of water shortage on plants.

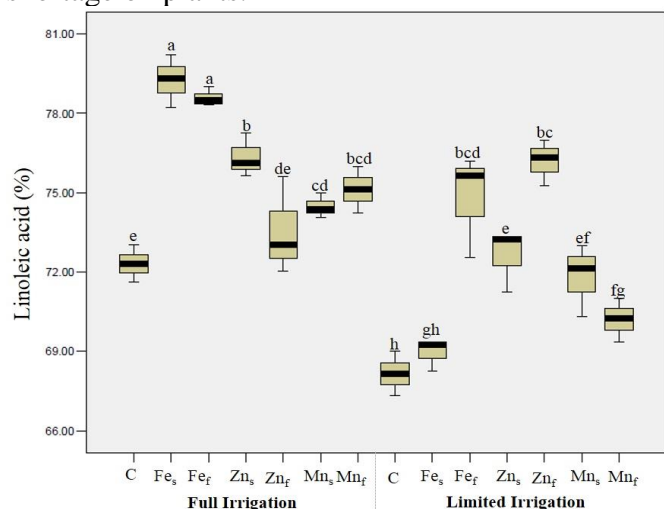


Fig. 3. Effect of fertilizers with micronutrient (Fe^{2+} , Zn^{2+} , Mn^{2+}) applied into soil or by spraying (indexes _s and _f, respectively) on stearic acid content in safflower seeds under different moisture regimes. Values with different superscripts are significantly different ($p < 0.05$). All values are mean \pm SEM.

Comparison of two moisture regimes indicated that the seed protein content of plants grown under the limited irrigation was higher than under full irrigation. Water scarcity is one of the most important factors that stimulates the degradation of photosynthetic proteins, such as RuBisCO, then can improve seed protein biosynthesis by increasing the remobilization of amino acids from source to sink (Distelfeld *et al.* 2014). Most of micronutrients can play a role as a cofactor in enzymes structure, hence enzyme activity largely depends on supply of micronutrients. In some cases, the stimulation of the remobilization of micronutrients from the source to the sink can affect the activity of the enzymes and, further, the qualitative aspects (Marschner 2012; Lemoine *et al.* 2013).

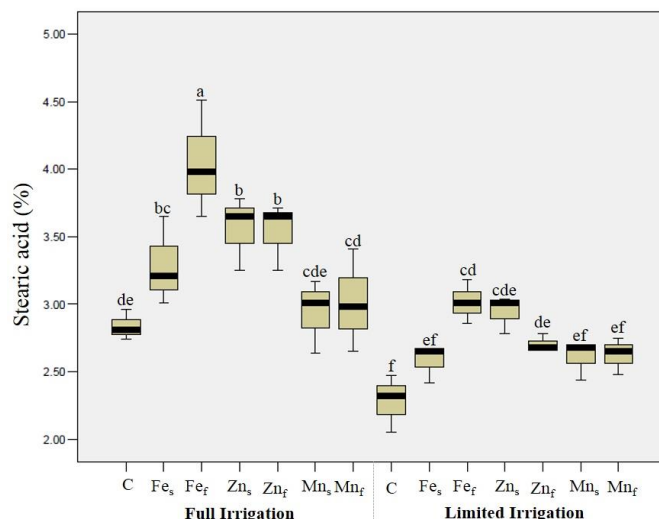


Fig. 4. Effect of fertilizers with micronutrient (Fe^{2+} , Zn^{2+} , Mn^{2+}) applied into soil or by spraying (indexes _s and _f, respectively) on linoleic acid content in safflower seeds under different moisture regimes. Values with different superscripts are significantly different ($P < 0.05$). All values are mean \pm SEM.

Most research pointed on negative impact of water deficit on photo-assimilates metabolism and phloem loading. Our results supported previous findings that water deficit strongly decreases biological yield, consequently production of fatty acids and other qualitative characteristics. In this regard, it has been reported that heat and drought stress alters the development of embryo and pericarp and result in reduction of oil content (Rondanini *et al.* 2003).

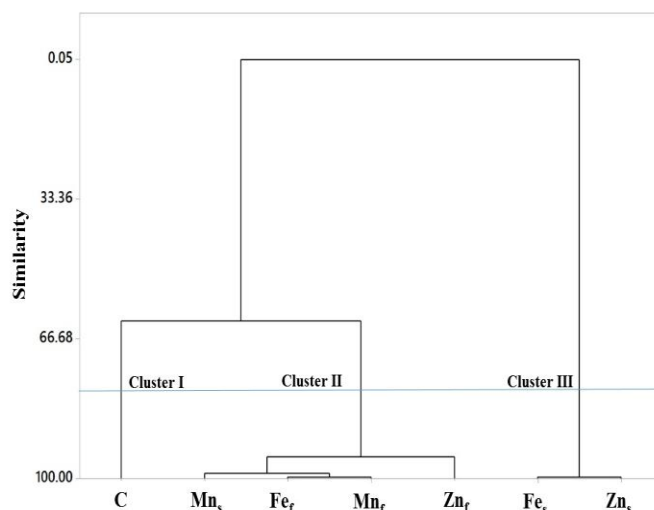


Fig. 5. Cluster analysis to classify quantitative and qualitative traits of safflower in terms of similarity in response to nutrients managements. For abbreviations, see Table 3.

The best growth and highest oil quality was recorded under growth in soil supplemented with

Fe^{2+} and Zn^{2+} . Comparison of Mn^{2+} content with other micronutrients in soil indicates that deficiencies of Fe^{2+} and Zn^{2+} are much more pronounced than Mn^{2+} . Zinc plays critical role as a cofactor in all enzymatic groups and, therefore can regulate the actions of a number of genes. Li *et al.* (2017) introduced a zinc-finger protein to soybean to modulate seed oil accumulation. Oil accumulation in seeds is associated with synthesis of fatty acids in plastid, and many key enzymes are involved in this process, including acetyl-CoA carboxylase and 3-ketoacyl-acyl carrier protein synthase. It appears that Zn is necessary for acetyl-CoA carboxylase function because a Zn-domain has been recognized for this enzyme. Also Fe as redox-active metal is involved in photosynthesis, mitochondrial respiration, nitrogen assimilation, phyto-hormone biosynthesis, protective process (Hänsch and Mendel 2009). Therefore, its supply can directly and indirectly affect the quality of the oil. It has been reported that eliminating plant nutrient deficiencies by improving plant growth and intracellular metabolic processes can lead to increase of seed oil percentage (Ravi *et al.* 2008; Movahhedy-Dehnavy *et al.* 2009).

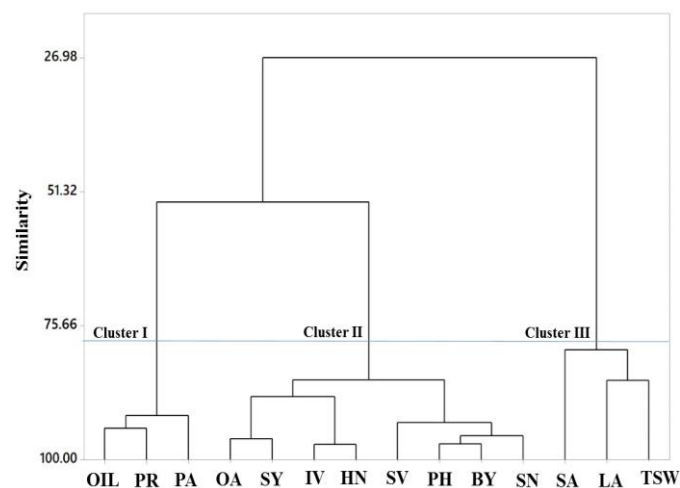


Fig. 6. Cluster analysis for classification of different fertilizer treatments in terms of similarity in influencing the studied traits in safflower. C: control (No-fertilizer application), micronutrient (Fe^{2+} , Zn^{2+} , Mn^{2+}) applied into soil or by spraying (indexes s and f , respectively).

Although some studies reported positive effects of foliar spray of micronutrient (Movahhedy-Dehnavy *et al.* 2009; Galavi *et al.* 2012; Janmohammadi *et al.* 2017), the results of this experiment showed that the soil application

of micronutrient was more efficient than foliar spray. This could be due to high temperature at the end of growing season, thickening of the cuticle, and low absorption of micronutrients.

Iodine value of oil is an index for determination of the unsaturation level in fatty acids. Water shortage significantly reduced Iodine value and this statue can be attributed to decreasing the amount or activity of desaturase enzymes (Movahhedy-Dehnavy *et al.* 2009). These results are consistent with those of other studies and suggest that drought stress decreases degree of fatty acid unsaturation and reduces the proportions of linolenic- (18:3) and linoleic (18:2) acids (Hamrouni *et al.* 2001). Saponification value of oil depends on the average molecular weight of the fatty acids constituent of fat. Saponification value has a direct relation with the amount short chain fatty acids. The high levels of this index, as a results of soil enrichment with Fe^{2+} and Zn^{2+} under full irrigation condition indicates to production and of the fatty acids and loading of fatty acids on glycerol backbone.

Conclusions

Although the safflower is relatively tolerant to drought stress, the water deficiency in the limited irrigation system causes a significant reduction in growth, yield components, oil content and qualitative aspects of the oil. Soil fertilization with Zn^{2+} and Fe^{2+} under full irrigation condition significantly increased the amount of oil content, palmitic acid, stearic acid and oil quality characteristics compared with the control. There was a significant correlation between seed weight and fatty acid with 18-carbon chain (stearic acid and linoleic acid). The treatments that improved the yield also often improved the quality of the seed oil. Although the foliar spray of micronutrients could increase some qualitative and quantitative properties compared with the control, the highest performance was achieved in soil application method. From the findings of present study it can be concluded that nutrition and irrigation managements are the important factors that affect the quality of oil in safflower balanced fertilization and accurate irrigation scheduling can greatly improve the quality of seed oil in semi-arid regions.

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