



## The effects of water stress and plant density on vegetative and reproductive characteristics of safflower in the semi-arid region

Hasan Kouchakkhani<sup>1</sup>, Mohsen Janmohammadi<sup>1</sup>,  
Naser Sabaghnia<sup>1</sup>

<sup>1</sup> Department of Plant Production and Genetics, Faculty of Agriculture,  
University of Maragheh, Maragheh, P.O. Box 55181-83111, Iran  
 **Corresponding author, E-mail: mohsen\_janmohammadi@yahoo.com**

Article history: Received 11 May 2024; Revised 03 November 2024;  
Accepted 12 November 2024; Available online 10 December 2024

©2024 Studia UBB Biologia. Published by Babeş-Bolyai University.



This work is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

**Abstract.** The limited availability of water for irrigation in semi-arid areas necessitates the use of deficit irrigation techniques. Deficit irrigation is an irrigation practice whereby water supply is reduced below maximum levels and a mild water-deficit stress affect the crop yield. Current field experiment was aimed to investigate different densities of safflower (*Carthamus tinctorius* L.) (33, 40, 50, 66 plants m<sup>2</sup> abbreviated as P<sub>33</sub>, P<sub>40</sub>, P<sub>50</sub>, and P<sub>66</sub>, respectively) under different soil moisture content (irrigation up to 100%, 70%, and 50% field capacity showed as FC<sub>100</sub>, FC<sub>70</sub>, and FC<sub>50</sub>, respectively) on the growth characteristics of safflower in a semi-arid region in the northwest of Iran. Number of days to maturity decreased significantly under deficit irrigation (FC<sub>70</sub> and FC<sub>50</sub>). The longest growth period was recorded for plants grown under P<sub>66</sub>-FC<sub>100</sub> conditions which was 7% higher than P<sub>50</sub>-FC<sub>100</sub>. The decrease in plant density caused an increase in chlorophyll content and this trend was more evident under FC<sub>100</sub>. Increasing the density under FC<sub>100</sub> conditions increased the plant height. However, this trend was not observed under deficit irrigation conditions. Decreasing the plant density per unit area induced the lateral growth, and increased the number of capitula per plant, the weight of capitulum, the number of achenes per capitulum and the 1000-achenes weight. This trend was more prominent under FC<sub>100</sub> conditions. Mild

deficit-irrigation (irrigation up to  $FC_{70}$ ) resulted approximately 20% reduction in achene yield. Thus, water management is very important to certify a conservational water supply in semi-arid regions. Irrigation up to  $FC_{70}$  under the studied area is a reasonable and good management method to save irrigation water.

**Keywords:** *Carthamus tinctorius* L., chlorophyll, deficit-irrigation, field capacity, phenology.

## Introduction

Safflower (*Carthamus tinctoriosus* L.) is an annual xerophilous plant from the Asteraceae family and native to semi-arid Mediterranean regions. Ancient surveys show that safflower is one the oldest crops and its cultivation dates back to the 12th century (Emongor, 2010). Although safflower is equipped to some characteristics such as deep root system and osmolytes accumulation to adapt to semi-arid areas, this plant sometimes classified as the forgotten or underutilized crops due to the lack of sufficient information about agronomic practices and slow breeding trends (La Bella *et al.*, 2018). Global warming and the occurrence of dry-spell in the growth season have been exacerbated by climate change during the recent decades, and this emphasizes the identification and use of drought-resistant plants in crop rotations (Janmohammadi and Sabaghnia, 2023a). Safflower has unique characteristics such as qualitative oil, forage, medicinal properties and it should be specifically considered in crop rotation in its original ecological zone (Kizil *et al.*, 2008; Abou Chehade *et al.*, 2022). Safflower is well adapted to crop rotation design, especially with cereals, and safflower insertion in rotation with cereals as dominant crops of semi-arid regions can break the lifecycle of cereal root diseases and improve ecological biodiversity (Hertel, 2016).

Adjusting the plant density is one of the most important factors influencing the achievement of the genetic potential of yield per unit area. However, the optimum plant density can be influenced by many other factors such as environmental conditions (soil type, depth of topsoil, readily available moisture, soil fertility, land slope, height above the sea level, air and soil temperatures, length of growth period), production systems and cultivar characteristics (Özaşık *et al.*, 2019). The robust structure and special anatomy of safflower to produce fertile lateral branches under low plant densities are among the things that should be considered in planting decisions. However, there is a compensatory

state between the achene yield components, and under the high plant densities, some flowers may remain sterile or produce low or smaller achenes (Elfadl *et al.*, 2009). However, there is still no comprehensive information about the response of safflower to plant densities and how to determine the optimal plant density.

On the other hand, the climate changes that happened during the last decades have aggravated the water shortage situation and the limitations of water resources prevailing in semi-arid regions (Janmohammadi and Sabaghnia 2023a). Therefore, the optimal use of available water resources and increasing the efficiency of water consumption are highly emphasized. Response of achene yield of safflower to plant densities is different in various soil moisture conditions. Deficit irrigation is a method of irrigation with predetermined less than usual volume of water, in which it is tried to improve the efficiency of water consumption (Pasandi *et al.*, 2014). Abd El-Lattief (2013) reported that the low-density plantings under deficit irrigation could produce an acceptable yield of safflower in the semi-arid region. High water consumption efficiency can be achieved by using precise deficit irrigation methods with a slight drop in seed yield. Sefaoğlu and Özer (2022) indicated that in planting safflower at different inter-row distances (20, 40, and 60 cm) along with various amounts of seed used (20, 40, and 60 kg ha<sup>-1</sup>) under fully irrigated conditions, the highest seed yield and oil content were obtained at high plant densities with low inter-row spacing. Also, increasing the density of safflower increased the leaf area duration (LAD), chlorophyll content, and increased the length of the development period (Moatshe *et al.*, 2020). Therefore, assessment of safflower response to water shortage and different planting densities can be very advantageous for in semi-arid areas. The present experiment aimed to evaluate the effect of planting density and different irrigation levels on the growth, agronomic, phenological characteristics, and yield components of safflower in the semi-arid region in the northwest of Iran.

## **Materials and methods**

### ***Site description***

The present experiment was carried out in the research farm of the Faculty of Agriculture, University of Maragheh, Maragheh, in North West of Iran (latitude 37°23' N, longitude 46°16' E and, height from sea level 1485 m) during the growing season of 2021-2022. Based on the Köppen-Geiger climate grouping, the region is cold and semi-arid in terms of climate and has predominant winter and spring rains (early and middle months). Some metrological characteristics during the safflower growing season in the Maragheh region in northwest Iran are shown in Tab. 1. Soil sampling was performed in the experimental field to determine

its physicochemical characteristics. The soil texture was clay loam consisting 41% clay, 37% silt and 22% sand. Some chemical soil characteristics were: pH= 7.51, electrical conductivity (EC) =1.14 dsm<sup>-1</sup>, organic matter = 0.69 g kg<sup>-1</sup>, nitrogen (N) = 0.082%, available phosphorus = 14.21 mg kg<sup>-1</sup> and available potassium (K) = 320 mg kg<sup>-1</sup>. The amount of annual potential evaporation and transpiration in the studied area was 1375 mm.

### ***Soil preparation and application of farmyard manure***

The primary tillage was done by a moldboard plow on November 2021, then 15 t ha<sup>-1</sup> of rotted farmyard manure was integrated with the topsoil by a disk and a rotary harrow. At the time of planting, 90 kg ha<sup>-1</sup> superphosphate and 50 of urea kg ha<sup>-1</sup> were utilized. In early March, secondary plowing was done and finally, ridging operations was done using the Mini Tiller-furrower. In order to prevent the loss of moisture stored in the final plowed soil or to prepare the seed bed, the minimum amount of soil was turned over. The experiment was conducted as a complete factorial experiment in split-plots with three replications. In this experiment, the effects of two factors (plant density and irrigation regimes) were investigated. Plant densities in four levels were: 33, 40, 50 and 66 plant m<sup>2</sup> (abbreviated as P<sub>33</sub>, P<sub>40</sub>, P<sub>50</sub>, and P<sub>60</sub>, respectively) which was created through various inter-row planting distances (30, 40, 50 and 60 cm). Irrigation regimes in three levels were: water supply up to 100%, 70% and 50% field capacity (abbreviated as FC<sub>100</sub>, FC<sub>70</sub> and FC<sub>50</sub>, respectively). The main plots were allocated to different plant densities (P<sub>33</sub>, P<sub>40</sub>, P<sub>50</sub>, and P<sub>60</sub>). Sub-plots were assigned to irrigation regimes (FC<sub>100</sub>, FC<sub>70</sub>, and FC<sub>50</sub>). Each experimental unit (secondary plot) had an area of 16 m<sup>2</sup> (4×4m) with 6-13 planting rows according to the different inter-row spacing. After completing the preparation of seedbed safflower achenes (Cv. Saffeh) were manually planted on an intra-row spacing of 5 cm at the depth of 3 cm on March 9. Immediately after planting, all plots were fully irrigated uniformly up to the field capacity.

### ***Irrigation treatments***

The following formula was used to calculate the water required to reach the field capacity (Hasanuzzaman *et al.*, 2016).  $RI = (SFC \times SM) \times BD \times RD$ . Where RI is the required irrigation (mm), SFC: is the selected field capacity percentage, SM: is soil moisture content before irrigation (through gravimetric method), BD: is soil bulk density g cm<sup>-3</sup>, RD: is the rooting depth (Average root development in the soil of the studied area). Soil water content was determined by a Time

Domain Reflectometry sensor (TDR 200, Campbell Scientific, Inc. USA) in three-day intervals. A pressure plate applied for measuring the soil moisture in suctions between 0.3 -15 bar and data were used for determine the irrigation schedules. A drip irrigation system with mainline polyethylene pipes was applied for irrigation. Irrigation conveyance and distribution efficiency was 95%. All treatments were irrigated primarily with an identical quantity of water (FC100) for three weeks to ensure ideal seed germination and seedling establishment. The irrigation was performed when half of the available water was depleted compared to the first days of the previous irrigation in each soil moisture condition. In order to prevent moisture leakage between the plots, one meter of margin was left uncultivated. Except for irrigation and plant density treatments, other agronomic managements were implemented uniformly on all experimental plots. Weed control was done manually at the same time as thinning and also during other growth stages.

### ***Plant growth measurements***

Monitoring the stages of plant growth was done regularly with daily inspection of the field. The stages of plant development were recorded based on reaching 70% of the plants in each plot to each stage of development (Flemmer *et al.*, 2015). The number of days to reach physiological maturity (BBCH: 87, 70% of the capitulum area yellow: fruits reach physiological maturity) was calculated by regularly visiting the field and counting the days from planting date. In the physiological maturity stage, the height of the ten plants was randomly measured using a meter from the soil surface to the tip of the main capitulum. Measurement of chlorophyll was measured through the portable chlorophyll meter (SPAD 502, Minolta, Japan) in the leaves above the canopy at the end stages of the development of the main capitulum at the top of the main shoot (BBCH scale= 61). The percentage of ground coverage by canopy was obtained visually by evaluating the amount of visible ground from the top of the canopy in each experimental unit. Ground cover was estimated during the final stages of reproductive growth when capitulum and fruits reach final size (BBCH scale= 79). Measurement of the diameter of the stem was performed in the early reproductive stages (capitulum emergence at the main stem) with a caliper. In the physiological maturity stage, after removing the marginal effects, 10 plants were randomly selected from the middle parts of the experimental plots, and the agronomic characteristics and yield components such as the number of capitula per plant, the mean diameter of the capitulum, achene number per main and secondary capitula, weight of capitula, thousand achene weight, achene number per secondary capitula, achene yield, and biological yield. Biological yield was determined by arbitrarily placing a 100 cm quadrat in central parts of each of the experimental units. After determining the harvesting area with quadrat, the plants

were harvested and dried in oven (72 °C for 48h), and the dry weight of the whole plant and the weight of the seeds were weighed. The harvest index was obtained through the ratio of achene yield to biological yield per unit area. The gathered data of evaluated traits were evaluated in terms of uniformity with Kolmogorov Smirnov and Bartlett's tests.

### ***Statistical data analysis***

*Data* statistically analyzed based on linear model procedure for a split-split plot design by SAS software (version 9.4). Means were separated using least significant differences test at 95% level of probability. Principal component analysis (PCA) and related statistic and graph provided by Minitab software (version 19.2). Box plots were drawn with Statistica software (version 13.0) to compare the average mutual effects of irrigation and plant density.

### **Results**

The evaluation of meteorological data showed that with the progress of plant development stages and approaching the summer season, the amount of precipitation decreased significantly, and on the other hand, the amount of actual evapotranspiration increased (Tab. 1).

**Table 1.** Meteorological characteristics during the safflower growing season in Maragheh region in northwest Iran

<b>Parameter</b>	<b>Jan</b>	<b>Feb</b>	<b>Mar</b>	<b>Apr</b>	<b>May</b>	<b>Jun</b>	<b>July</b>	<b>Aug</b>
Maximum temperature (C°)	2.1	9.9	15.9	24.7	30.5	35.3	37.4	32.2
Minimum temperature (C°)	-5.7	-0.3	5.2	11.4	15.3	21.7	22.5	19.7
Air humidity (%)	68	63.4	46.3	41.5	42.8	27.5	32.4	26.4
Precipitation (mm)	21.6	9.1	34.9	10.7	1.2	0.5	0.2	%0.0
Actual evapotranspiration (mm)	27.41	35.24	56.72	78.86	98.49	108.51	127.41	134.65

### ***Evaluation of growth characteristics***

The evaluation of one of the phenological characteristics of the plant, i.e. the number of days to reach physiological maturity, showed that the mutual effects of irrigation regime and plant density on this component are significant ( $p \leq 0.05$ ). The longest growth period for plants grown under favorable conditions of irrigation (FC<sub>100</sub>) and high density (P<sub>66</sub>) was recorded with about 151 days. However, reducing the density under optimal moisture conditions (FC<sub>100</sub>-P<sub>33</sub>)

reduced the number of days to maturity by 22 days. The shortest length of the growth period was recorded in FC<sub>50</sub> conditions and no difference in days to maturity was observed between different planting densities. In FC<sub>70</sub> irrigation conditions, reducing the density from P<sub>66</sub> to P<sub>33</sub> accelerated the number of days to maturity by about 14% (Tab. 2).

**Table 2.** Effect of different soil moisture regimes and plant density (inter-row spacing) on morphological and growth characteristics of safflower (*Carthamus tinctorius* L.) in the semi-arid area of Iran.

	Treatments	DTM	PH	TBN	SD	GCP	BBY
Soil moisture regimes	FC <sub>100</sub>	137.66 <sup>a</sup>	72.66 <sup>a</sup>	30.75 <sup>a</sup>	11.59 <sup>a</sup>	52.20 <sup>a</sup>	4662.40 <sup>a</sup>
	FC <sub>70</sub>	118.91 <sup>b</sup>	63.41 <sup>b</sup>	22.50 <sup>b</sup>	10.30 <sup>b</sup>	43.80 <sup>b</sup>	3613.92 <sup>b</sup>
	FC <sub>50</sub>	102.34 <sup>c</sup>	49.44 <sup>c</sup>	16.66 <sup>c</sup>	10.29 <sup>b</sup>	34.40 <sup>c</sup>	2981.25 <sup>c</sup>
Plant density	P <sub>33</sub>	127.17 <sup>a</sup>	58.44 <sup>c</sup>	18.88 <sup>d</sup>	8.36 <sup>d</sup>	38.66 <sup>d</sup>	3313.33 <sup>d</sup>
	P <sub>40</sub>	121.73 <sup>b</sup>	59.72 <sup>bc</sup>	22.22 <sup>c</sup>	10.16 <sup>c</sup>	42.26 <sup>c</sup>	3525.04 <sup>c</sup>
	P <sub>50</sub>	117.60 <sup>c</sup>	63.26 <sup>ab</sup>	24.77 <sup>b</sup>	11.32 <sup>b</sup>	44.80 <sup>b</sup>	3790.84 <sup>b</sup>
	P <sub>66</sub>	112.04 <sup>d</sup>	65.93 <sup>a</sup>	27.33 <sup>a</sup>	13.06 <sup>a</sup>	48.12 <sup>a</sup>	4380.87 <sup>a</sup>
Statistical significance							
	S	**	**	**	ns	*	**
	P	**	**	**	**	**	**
	S*P	**	*	ns	ns	ns	*
	CV	6.01	9.88	7.34	10.14	4.62	4.27

FC: Irrigation amount according to field capacity, P<sub>33</sub>-P<sub>66</sub>: Plant density per square meter with 30 cm to 60 cm inter-row spacing. DTM: day to maturity, PH: plant height (cm), TBN: total branch numbers, SD: stem diameter (mm), GCP: ground cover percentage by plant canopy, BBY: biological weight (kg ha<sup>-1</sup>). CV: coefficient of variation. Values in a column with the same letter (s) do not have a statistically significant difference, whereas values with dissimilar letters are statistically different. ns = not significant, \* = significant at 5% level of probability, \*\* = significant at 1% level of probability.

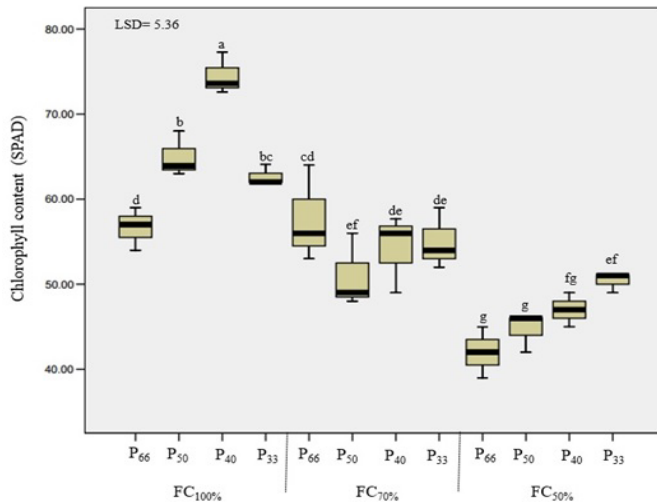
The tallest plants were recorded in FC<sub>100</sub> P<sub>66</sub> conditions and reducing the density by half (P<sub>33</sub>) caused a 24% decrease in plant height. The reducing effect of density on the height was clear only under favorable irrigation conditions, and under deficit irrigation conditions (FC<sub>50</sub> and FC<sub>70</sub>), the effect of density on the height was not significant. Only the adoption of high planting densities in the conditions of moderate irrigation (FC<sub>70</sub>P<sub>66</sub>) caused a slight increase in the

height of the plant. With the decrease in density, the height of the first capitulum on the plant from the ground level also decreased.

The lowest number of lateral branches was recorded in FC<sub>50</sub>P<sub>66</sub> with 13 branches. Reduction of density in all irrigation regimes reduced the number of lateral branches. The highest number of side branches (35 branches) was recorded under FC<sub>100</sub>P<sub>33</sub> conditions. The effect density on the number of side branches was more evident under favorable irrigation conditions (Tab. 2). Reducing density from P<sub>66</sub> to P<sub>33</sub> increased the number of lateral branches by 44%.

The evaluation of the stem diameter showed that this vegetative component was not significantly affected by the irrigation regime. However, the increase in plant density per unit area greatly reduced the stem diameter, the lowest stem diameter was under P<sub>66</sub>FC<sub>50</sub> conditions and P<sub>66</sub>FC<sub>70</sub> and the maximum stem diameter was recorded in FC<sub>100</sub>P<sub>33</sub> conditions.

The evaluation of the chlorophyll content showed that the decreasing the soil moisture caused a significant decrease in this pigment. A 30% and 50% reduction in irrigation resulted in a 15% and 29% decrease in the chlorophyll content, respectively (Fig. 1). On the other hand, decreasing the density from P<sub>66</sub> to P<sub>50</sub>, P<sub>40</sub> and P<sub>33</sub> increased chlorophyll by 3%, 12% and 8%, respectively. The highest amount of chlorophyll was recorded under FC<sub>100</sub> P<sub>40</sub>, while the plants grown in FC<sub>50</sub> P<sub>66</sub> showed the lowest amount of chlorophyll.



**Figure 1.** Mean comparison of chlorophyll content of the upper leaves of safflower plants cultivated in different densities and under different soil moisture regimes in the semi-arid region in the northwest of Iran. In each box, the horizontal dashed line represents the mean. FC: Irrigation amount according to field capacity, P<sub>33</sub>-P<sub>66</sub>: Plant density per square meter with 30 cm to 60 cm inter-row spacing. Boxes with different letters have statistically significant differences ( $p < 0.05$ ). *LSD*: least significant difference.



Estimation of ground canopy cover showed that the mutual effects of irrigation regime and density on this trait was significant at the statistical level of 5%. The highest ground canopy cover was recorded under FC<sub>100</sub> and P<sub>66</sub> conditions. The lowest amount of ground canopy cover was observed in densities P<sub>33</sub> and P<sub>40</sub> under FC<sub>50</sub> (38%). Although the increase in the density increased the ground canopy cover, this trend was more noticeable under favorable irrigation conditions.

The evaluation of biological yield showed that a 30% and 50% reduction in irrigation resulted in a 23% and 36% decrease in the biological yield, respectively. The trend of decreasing biological yield with decreasing density was more evident in FC<sub>70</sub> conditions. The biological yield under FC<sub>70</sub> conditions decreased from 4216 kg ha<sup>-1</sup> to 3221 kg ha<sup>-1</sup> by decrease of plant density from P<sub>66</sub> to P<sub>33</sub>.

### Achene yield components

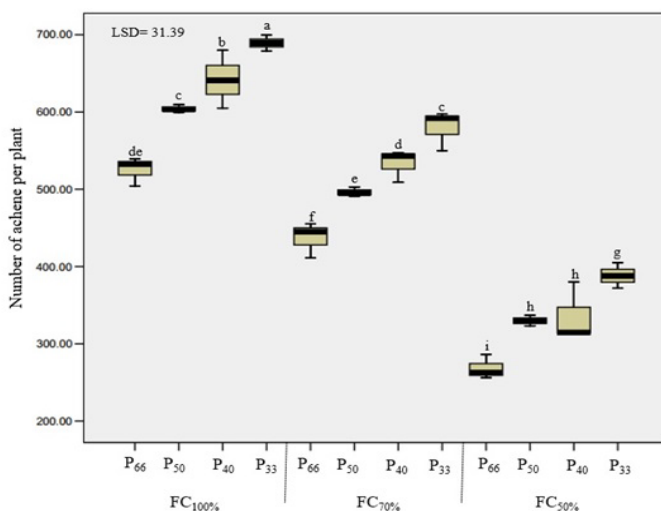
The effects of soil moisture regimes and plant densities are summarized in Tab. 3.

**Table 3.** Impact of water stress and different inter-row spacing on achene yield component of safflower (*Carthamus tinctorius* L.) in northwest of Iran.

	Treatments	TCN	TCW	ANM	ANS	CMD	HI
Soil moisture regimes	FC <sub>100</sub>	30.33 <sup>a</sup>	48.87 <sup>a</sup>	354.83 <sup>a</sup>	260.40 <sup>a</sup>	2.82 <sup>a</sup>	22.31 <sup>a</sup>
	FC <sub>70</sub>	23.33 <sup>b</sup>	34.34 <sup>b</sup>	301.00 <sup>b</sup>	210.60 <sup>b</sup>	2.46 <sup>b</sup>	20.17 <sup>b</sup>
	FC <sub>50</sub>	17.00 <sup>c</sup>	25.22 <sup>c</sup>	193.73 <sup>c</sup>	136.94 <sup>c</sup>	2.00 <sup>c</sup>	21.52 <sup>a</sup>
Plant density	P <sub>33</sub>	29.44 <sup>a</sup>	42.03 <sup>a</sup>	314.66 <sup>a</sup>	237.77 <sup>a</sup>	2.67 <sup>a</sup>	20.18 <sup>a</sup>
	P <sub>40</sub>	24.00 <sup>b</sup>	39.28 <sup>a</sup>	293.85 <sup>b</sup>	209.92 <sup>b</sup>	2.44 <sup>b</sup>	20.18 <sup>a</sup>
	P <sub>50</sub>	22.11 <sup>c</sup>	34.37 <sup>b</sup>	275.90 <sup>c</sup>	200.97 <sup>b</sup>	2.40 <sup>b</sup>	21.67 <sup>a</sup>
	P <sub>66</sub>	18.66 <sup>d</sup>	28.88 <sup>c</sup>	248.33 <sup>d</sup>	161.91 <sup>c</sup>	2.44 <sup>b</sup>	21.66 <sup>a</sup>
Statistical significance							
	S	**	**	**	**	**	**
	P	**	**	**	**	**	Ns
	S*P	*	ns	ns	ns	**	**
	CV	6.43	8.67	5.51	6.92	3.77	5.44

FC: Irrigation amount according to field capacity, P<sub>33</sub>-P<sub>66</sub>: Plant density per square meter with 30 cm to 60 cm inter-row spacing. TCN: total number of capitula per plant, TCW: weight of capitula per plant (g), ANM: achene number per main capitula, ANS: achene number per secondary capitula, CMD: diameter of capitulum (cm), HI: harvest index (%). CV: coefficient of variation. Values in a column with the same letter (s) do not have a statistically significant difference, whereas values with dissimilar letters are statistically different. ns = not significant, \* = significant at 5% level of probability, \*\* = significant at 1% level of probability.

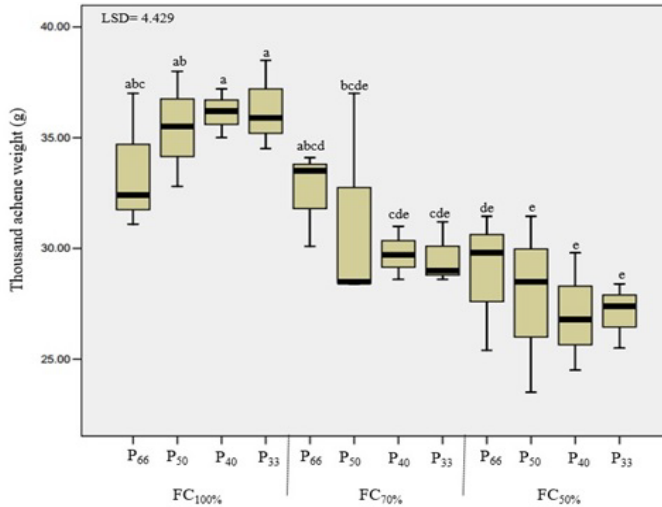
The number of capitulum per plant (TNC) decreased with the decline in soil moisture, so that with a 30% and 50% decrease in water supply compared to FC<sub>100</sub>, TNC decreased by 23% and 44%. Although the reduction of plant density per unit area in all irrigation regimes increased number of capitula per plant, this increase was significant in FC 100 conditions (Tab. 3). The highest number of capitula per plant was recorded under FC<sub>100</sub> P<sub>33</sub> (38.26) and the lowest number under FC<sub>50</sub> P<sub>66</sub> (12.60). A relatively similar trend was observed for the weight of the capitulum, and the decrease of 30% and 50% of irrigation compared to FC<sub>100</sub> caused a decrease of 30% and 48% of the weight of the capitulum. Seed planting in rows with 60 cm intervals (P<sub>33</sub>) increased the capitulum weight by 45% compared to dense planting at 30cm row spacing (P<sub>66</sub>). Total achene number (TAN) in the plant was strongly affected by irrigation regimes and under FC<sub>70</sub> and FC<sub>50</sub> conditions it decreased by 17% and 46% compared to optimal irrigation conditions. On the other hand, with a decrease in plant density from P<sub>66</sub> to P<sub>50</sub>, P<sub>40</sub> and P<sub>33</sub> TAN showed an increase of 16%, 22.7% and 34% respectively. The highest TAN was recorded under FC<sub>100</sub> P<sub>33</sub> with 689 achenes and the lowest TAN was related to plants grown under FC<sub>50</sub> P<sub>66</sub> with 268.2 achenes (Fig. 2).



**Figure 2.** The effect of different safflower planting densities and different irrigation regimes on number of achenes per plant. FC: Irrigation amount according to field capacity, P<sub>33</sub>-P<sub>66</sub>: Plant density per square meter with 30 cm to 60 cm inter-row spacing. Boxes with different letters have statistically significant differences ( $p < 0.05$ ). *LSD*: least significant difference.

The mutual effects of irrigation and density were significant at the 1% level on the capitulum diameter. In all moisture regimes, planting in wide rows (P<sub>33</sub>) significantly increased the capitulum diameter. under FC<sub>100</sub> conditions, the lowest capitulum diameter was recorded in P<sub>66</sub> (2.41 cm) and reducing plant density to P<sub>50</sub>, P<sub>40</sub>, and P<sub>33</sub> increased capitulum diameter by 19%, 23%, and 29%. However, plants grown under deficit irrigation conditions showed the

significant increase in capitulum diameter only at very low plant densities ( $P_{33}$ ). The investigation of thousand achenes weight (TAW) indicated a significant effect of irrigation regime on this component. A decrease of 30% and 50% of irrigation compared to optimal irrigation conditions led to a decrease of 12% and 21% of TAW. The lowest amount of TAW was recorded under severe deficit irrigation ( $FC_{50}$ ) and the highest amount of TAW was recorded under  $FC_{100}$  with densities of  $P_{33}$  and  $P_{40}$  (Fig. 3).

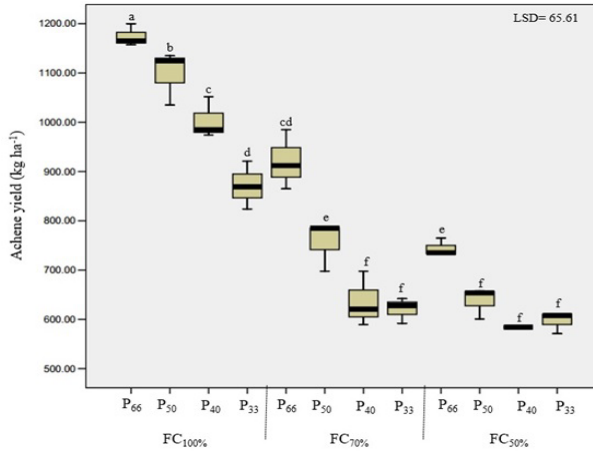


**Figure 3.** Investigating the effect of different planting densities under different soil moisture conditions on the 1000- achene weight of safflower in the semi-arid region of northwestern Iran. FC: Irrigation amount according to field capacity,  $P_{33}$ - $P_{66}$ : Plant density per square meter with 30 cm to 60 cm inter-row spacing. Boxes with different letters have statistically significant differences ( $p < 0.05$ ). *LSD*: least significant difference.

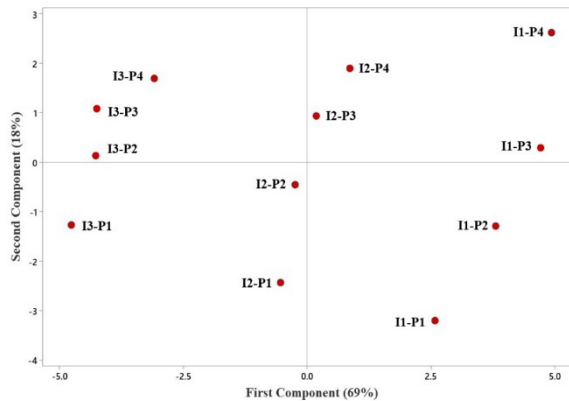
Achene yield per unit area was strongly influenced by the investigated treatments and the mutual effects of irrigation regime and plant density were significant on this trait. With a 30% and 50% decrease in irrigation water supply, achene yield decreased by 29% and 38%. However, the increase in plant density significantly increased achene yield only under  $FC_{100}$  and  $FC_{70}$  conditions. Reducing the plant density up to  $P_{33}$  under optimal irrigation conditions caused a 25% decrease in achene yield, while planting in low plant densities under  $FC_{70}$  decreased achene yield by 33%. However, the reduction of plant density under severe deficit irrigation reduced achene yield by 21%, but no difference between achene yield was observed between  $P_{33}$ ,  $P_{40}$  and  $P_{50}$  densities (Fig. 4).

Principal component analysis (PCA) provided the possibility of summarizing the data and the first component was able to distinguish the irrigation regimes from each other and the best safflower performance was obtained under the

optimal moisture condition (FC<sub>100</sub>) and under mild deficit irrigation (FC<sub>70</sub>). On the other hand, the second component was able to distinguish the high plant densities (P<sub>66</sub> and P<sub>50</sub>) that produced the highest achene yield (Fig. 5). The results of PCA showed that high densities even under mild deficit irrigation conditions can be a suitable solution for acceptable safflower production in a semi-arid region.



**Figure 4.** The effect of different planting densities and different irrigation regimes on safflower achene yield. FC: Irrigation amount according to field capacity, P<sub>33</sub>-P<sub>66</sub>: Plant density per square meter with 30 cm to 60 cm inter-row spacing. Boxes with different letters have statistically significant differences ( $p < 0.05$ ). *LSD*: least significant difference.



**Figure 5.** Plot of the first two principal component (PC) scores, PC1 vs. PC2 related to the distribution of different combined treatments (plant density and soil moisture regime) and their similarity in influencing the evaluated agronomic traits. FC: Irrigation amount according to a proportion of field capacity, P<sub>33</sub>-P<sub>66</sub>: Plant density per square meter with 30 cm to 60 cm inter-row spacing

## Discussion

The trend of temperature changes and the amount of precipitation during the growing season in the studied area indicates that the amount of soil moisture loss through evaporation and transpiration is very high. In such a situation, it seems that rainfed farming cannot have high success and the use of irrigation is necessary to achieve acceptable yields. Although safflower is a plant with relatively good adaptability to water deficit conditions and can guarantee its survival and production by self-regulating its growth in different densities, the results obtained in this experiment showed that it was strongly affected by moisture conditions and densities. The low quality of the soil such as low permeability and low water holding capacity due to the low organic matter of the soil and the application of improper crop rotations and tillage systems as well as the intensification of climate change in semi-arid areas are among the possible reasons for strong response of safflower to moisture conditions and plant density in the mentioned area (Janmohammadi and Sabaghnia, 2023a).

Plant phenology was affected by irrigation regimes and densities. The length of the plant growth period can be strongly influenced by environmental conditions (temperature, humidity, soil condition, length of the growing season in the region, etc.) and plant density (Caliskan and Caliskan, 2018). Under favorable environmental conditions, increasing the density can accelerate flowering and maturity by increasing the speed of growth and development (Moatshe *et al.*, 2020). However, it appears that the moisture content of the soil in mild and severe deficit irrigation conditions ( $FC_{70}$  and  $FC_{50}$ ) due to the continuation of deficit irrigation conditions and lack of sufficient rainfall during the growth period has largely become yield-reducing drought stress. Response of yield components to water stress were somewhat different. Studies have shown that there is a tight correlation between the number of capitula and achene yield (Janmohammadi *et al.*, 2016; Sefaoğlu and Özer, 2022; Janmohammadi and Sabaghnia, 2023; Fatthi *et al.*, 2024). However, increasing plant density seems to have several pros and cons depending on environmental conditions. In the present experiment, the number of lateral branches and the number of capitula increased significantly by reducing the plant density per unit area. However, due to the presence of negative and compensatory relationships between yield components and the limited capacity of the plant to produce capitula, the increase of some of the plant components in the conditions of low densities could not compensate the low plant numbers and the yield did not show a significant increase. Contrary to the findings of Ehsanzadeh and Zareian Baghdad-Abadi (2003) we did not find any improving effects from low density. These researchers reported that the use of low densities of 16 and 22 plants  $m^{-2}$  through 50 cm inter-

row spacing and 9-12 cm intra-row spacing under well-irrigated conditions in the semi-arid region showed the best performance compared to high densities caused by the reduction of intra-row spacing. In any case, according to the humidity limits and soil conditions of the studied area, increasing the number of plants in the intra-row can increase intra-species competition. One of the important factors affecting the performance of achene is the leaf surface index. Usually, leaf area index of about 3-5 is necessary to produce maximum dry matter (Steberl *et al.*, 2019). The obtained results showed that high planting densities in the FC<sub>100</sub> and FC<sub>70</sub> irrigation regimes could increase the ground cover by the canopy, and this can be attributed to the increase in the leaf area index. The increase in the leaf area increases the absorption of solar radiation and subsequently improves the production of dry matter by photosynthesis. The findings showed that the source-sink relationship in safflower plants impacted by both agronomic management and external environmental factors. As was anticipated, in very high densities, the amount of dry matter production in a distinct plant decreased probably due to shading and the reduction of the photosynthesis rate of the lower leaves of the canopy.

Under semi-arid region it is very important the quickly covering the soil surface by the canopy to prevent the loss of soil moisture through evaporation and also to reduce the invasion of weeds (El-Beltagi *et al.*, 2022) therefore the planting in narrow rows with 30 cm inter-row spacing seems to be more justifiable. However, modification of the leaf area index through changing the plant density can affect the durability of the leaf area, chlorophyll content and the efficiency of photosynthesis per unit of leaf area. The results showed that under well-irrigated conditions (FC<sub>100</sub>) the chlorophyll content of leaves increased with decreasing plant densities, still under deficit irrigation conditions, plant density did not have much effect on chlorophyll content. Probably, under deficit irrigation, the drought stress caused the destruction and inhibition of chlorophyll synthesis (de Almeida Silva *et al.*, 2023). The study showed that the seed weight was less affected by the studied treatments. This trait is strongly influenced by genetic characteristics and is less influenced by environmental factors (Licata *et al.*, 2023).

In this experiment, plant densities did not have a significant effect on seed weight. With the increase in density under favorable moisture conditions, probably due to the increase in inter-plant competition, the yield components decreased to some extent (Fasoula and Tollenaar, 2005). But the increase in the number of plants was able to compensate for the loss caused by the competition between plants and the total yield in unit of area increased (Zheng *et al.*, 2021). However, increasing the density of plants per unit area can affect access to needed resources such as water, nutrients, and light (Chen *et al.*, 2022), so conscious

and accurate fertilizer management, improving soil permeability through the use of animal manures and plant residues can be effective. There was 20% decrease in achene yield under mild deficit irrigation conditions (FC<sub>70</sub>) compared to well-irrigated conditions. However, by considering the positive and negative attributes of arguments and due to the water scarcity in the studied area and also the coincidence of safflower growth with the cultivation of other cash crops, the use of this deficit irrigation technique is still recommended.

## Conclusions

In total, the reduction of irrigation volume reduced the growth and all yield components. Reducing the irrigation amount by 50% accelerated the maturity of plants. Growth characteristics and yield components under deficit irrigation conditions did not significantly affect by planting densities. Applying low plant density under the well-irrigated or mild deficit irrigation (FC<sub>70</sub>) conditions led to a considerable increase in lateral growth of plants. However, increased plant lateral growth at low planting densities could not compensate for the decrease in achene yield caused by the low plant and capitula number per unit area. Applying the 30 cm inter-row spacing with mild deficit irrigation produced an acceptable achene yield (900 kg ha<sup>-1</sup>) and led to significant water saving (30%). By utilizing narrow row (P<sub>33</sub>) and cover of the soil surface the evaporation reduced and most of the sun's radiation is received by the canopy. However, it is not recommended to use the deficit irrigation throughout the crop cycle. Because results showed that decrease in soil moisture during the reproductive period led to the reduction in the number of fertile branches, decreased length of the development period, and reduced the achene yield. Applying deficit irrigation during the vegetative growth period and providing an optimal level of water (FC<sub>100</sub>) during the sensitive reproductive phase may improve the efficiency of water use.

**Acknowledgements.** This research is a part of the doctoral thesis and we are grateful to University of Maragheh and especially the Department of Postgraduate Education for financial support.

## References

- Abd El-Lattief, E.A. (2013). Safflower yields and water use efficiency as affected by irrigation at different soil moisture depletion levels and plant population density under arid conditions. *Arch. Agron. Soil Sci.*, 59(11), 1545-1557.  
<https://doi.org/10.1080/03650340.2012.735769>

- Abou Chehade, L., Angelini, L.G. & Tavarini, S. (2022). Genotype and seasonal variation affect yield and oil quality of safflower (*Carthamus tinctorius* L.) under Mediterranean conditions. *Agronomy*, 12(1), 122. <https://doi.org/10.3390/agronomy12010122>
- Caliskan, S. & Caliskan, M.E. (2018). Row and plant spacing effects on the yield and yield components of safflower in a mediterranean-type environment. *Turk J Field Crops*, 23(2), 85-92. <https://doi.org/10.17557/tjfc.467442>
- Chen, J., Wang, Y., Zhi, X., Qiu, Y., Han, Y., Feng, L., Wang, Z., Li, X., Lei, Y., Xiong, S. & Wang, G (2022). Modifying the planting density to change water utilization in various soil layers and regulate plant growth and yield formation of cotton. *Field Crops Res.*, 289, 108738. <https://doi.org/10.1016/j.fcr.2022.108738>
- de Almeida Silva, M., Santos, H. L., de Sousa Ferreira, L., Silva, D. M. R., dos Santos, J.C.C. & de Almeida Prado Bortolheiro, F. P. (2023). Physiological changes and yield components of safflower (*Carthamus tinctorius* L.) lines as a function of water deficit and recovery in the flowering phase. *Agriculture*, 13(3), 558. <https://doi.org/10.3390/agriculture13030558>
- Ehsanzadeh, P. & Zareian Baghdad-Abadi, A. (2003). Yield, yield components and growth characteristics of two safflower genotypes under varying plant densities. *J. Crop Prod. Proc.*, 7 (1):129-140
- El-Beltagi, H.S., Basit, A., Mohamed, H.I., Ali, I., Ullah, S., Kamel, E.A., Shalaby, T.A., Ramadan, K.M., Alkhateeb, A.A. & Ghazzawy, H.S. (2022). Mulching as a sustainable water and soil saving practice in agriculture: A review. *Agronomy*, 12(8), 1881. <https://doi.org/10.3390/agronomy12081881>
- Elfadl, E., Reinbrecht, C., Frick, C. & Claupein, W. (2009). Optimization of nitrogen rate and seed density for safflower (*Carthamus tinctorius* L.) production under low-input farming conditions in temperate climate. *Field Crops Res.*, 114(1), 2-13.
- Emongor, V. (2010). Safflower (*Carthamus tinctorius* L.) the underutilized and neglected crop: a review. *Asian J. Plant Sci.*, 9(6), 299-306. <https://doi.org/10.3923/ajps.2010.299.306>
- Fasoula, V. A. & Tollenaar, M. (2005). The impact of plant population density on crop yield and response to selection in maize. *Maydica*, 50 (1), 39-48.
- Fatthi, M., Janmohammadi, M. & Sabaghnia, N. (2024). The agronomic response of *carthamus tinctorius* to sulphur fertilizers. *Agriculture and Forestry*, 70 (1): 203-215. <https://doi.org/10.17707/AgricultForest.70.1.14>
- Flemmer, A.C., Franchini, M.C. & Lindström, L.I. (2015). Description of safflower (*Carthamus tinctorius*) phenological growth stages according to the extended BBCH scale. *Ann. Appl. Biol.*, 166(2), 331-339. <https://doi.org/10.1111/aab.12186>
- Hasanuzzaman, M.D., Shabala, L. Brodribb, T.J., Zhou, M. & Shabala, S. 2016. Assessing the suitability of various screening methods as a proxy for drought tolerance in barley. *Funct. Plant Biol.*, 44(2): 253-266.
- Janmohammadi, M. & Sabaghnia, N. (2023a). Strategies to alleviate the unusual effects of climate change on crop production: a thirsty and warm future, low crop quality. A review. *Biologija*, 69(2): 121-133. <https://doi.org/10.6001/biologija.2023.69.2.1>



- Janmohammadi, M. & Sabaghnia, N. (2023b). Effects of foliar spray of nano-micronutrient and growth regulators on safflower (*Carthamus tinctorius* L.) performance. *Plant Nano Biology*, 5, 100045. <https://doi.org/10.1016/j.plana.2023.100045>
- Janmohammadi, M., Amanzadeh, T., Sabaghnia, N. & Ion, V. (2016). Effect of nano-silicon foliar application on safflower growth under organic and inorganic fertilizer regimes. *Bot. Lith.*, 22(1), 53-64. <https://doi.org/10.1515/botlit-2016-0005>
- Hertel, K. (2016) Tactical agronomy of safflower and linseed: place in the rotation, yield potential, time of sowing, plant growth and marketing. <https://grdc.com.au/Research-and-Development/GRDC-Update-Papers/2016/03/Tactical-agronomy-of-safflower-and-linseed>
- Kizil, S., Çakmak, Ö., Kirici, S. A. L. İ. H. A. & İnan, M. (2008). A comprehensive study on safflower (*Carthamus tinctorius* L.) in semi-arid conditions. *Biotechnol. Biotechnol. Equip.*, 22(4), 947-953. <https://doi.org/10.1080/13102818.2008.10817585>
- La Bella, S., Tuttolomondo, T., Lazzeri, L., Matteo, R., Leto, C. & Licata, M. (2019). An agronomic evaluation of new safflower (*Carthamus tinctorius* L.) germplasm for seed and oil yields under Mediterranean climate conditions. *Agronomy*, 9(8), 468. <https://doi.org/10.3390/agronomy9080468>
- Licata, M., Farruggia, D., Iacuzzi, N., Matteo, R., Tuttolomondo, T. & Di Miceli, G. (2023). Effects of genotype and climate on productive performance of high oleic *Carthamus tinctorius* L. under rainfed conditions in a semi-arid environment of Sicily (Italy). *Plants*, 12(9), 1733. <https://doi.org/10.3390/plants12091733>
- Moatshe, O. G., Emongor, V. E., Balole, T. V. & Tshwenyane, S. O. (2020). Safflower genotype by plant density on yield and phenological characteristics. *Afr. Crop Sci. J.* 28(s1), 145-163. <https://dx.doi.org/10.4314/acsj.v28i1.11S>
- Özaşık, İ., Kaya, M. D. & Kulan, E. G. (2019). The optimum plant density for vigorous seed production in safflower. *Turkish Journal of Agriculture - Food Science and Technology*, 7(2), 301-305. <https://doi.org/10.24925/turjaf.v7i2.301-305.2275>
- Pasandi, M., Janmohammadi, M. & Karimizadeh, R. 2014. Evaluation of genotypic response of Kabuli chickpea (*Cicer arietinum* L.) cultivars to irrigation regimes in Northwest of Iran. *Agriculture (Pol'nohospodárstvo)*, 60(1): 22-30. <https://doi.org/10.2478/agri-2014-0003>
- Sefaoğlu, F. & Özer H. (2022). Response of Safflower (*Carthamus tinctorius* L.) to Planting Rate and Row Spacing in a High Altitude Environment. *Erciyes Tarım ve Hayvan Bilimleri Dergisi*, 5(1), 1-10. 10.55257/ethabd.1090609
- Steberl, K., Boote, K. J., Munz, S. & Graeff-Hönniger, S. (2019). Modifying the CROPGRO safflower model to simulate growth, seed and floret yield under field conditions in Southwestern Germany. *Agronomy*, 10(1), 11. <https://doi.org/10.3390/agronomy10010011>
- Zheng, B., Zhang, X., Wang, Q., Li, W., Huang, M., Zhou, Q. & Jiang, D. (2021). Increasing plant density improves grain yield, protein quality and nitrogen agronomic efficiency of soft wheat cultivars with reduced nitrogen rate. *Field Crops Res.*, 267, 108145. <https://doi.org/10.1016/j.fcr.2021.108145>

