

## EVALUATION OF THE PHOTOSYNTHETIC PIGMENTS AND GROWTH OF *CARTHAMUS TINCTORIUS* UNDER DIFFERENT SOIL MOISTURE

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**Abstract:** Climatic shifts, the occurrence of dry and low-precipitation spells, and inappropriate fertilizer management in semi-arid areas are the main reasons for low safflower yield. Current experiment aimed to evaluate the different irrigation regimes (I<sub>1</sub>: rainfed or no-irrigated condition, I<sub>2</sub>: one-time irrigation after seeds planting, I<sub>3</sub>: supplementary irrigation in the flowering stage, I<sub>4</sub>: supplementary irrigation in the flowering and seed set stages, I<sub>5</sub>: well-irrigated conditions) and the use of different fertilizers (C: control or non-use of fertilizers, NPK: chemical nitrogen, phosphorus, and potassium, FYM: 20 t ha<sup>-1</sup> animal manure) in the high and semi-arid region of Hashtroud. The results showed that the highest amounts of chlorophyll *a* (39.33 mg ml<sup>-1</sup>) and chlorophyll *b* (18.3 mg ml<sup>-1</sup>) were recorded under I<sub>5</sub>+ NPK conditions and the lowest was related to plants grown under I<sub>1</sub>+ C conditions. The highest xanthophylls was recorded for plants grown under I<sub>2</sub>+FYM (0.79 mg ml<sup>-1</sup>), and I<sub>3</sub>+NPK (0.72 mg ml<sup>-1</sup>) conditions, and the lowest amount was related to I<sub>5</sub>+NPK conditions (0.17 mg ml<sup>-1</sup>). The tallest plants (160.78 cm), the largest canopy span (68.66 cm), the largest head diameter (33 mm), and the largest number of heads per plant (26.2) were recorded under I<sub>5</sub>+ NPK conditions. The highest 1000-seeds were observed under I<sub>3</sub>+C conditions (27.66 g) and the lightest seeds (25.16 g) were obtained from plants grown under I<sub>4</sub>+NPK conditions. The use of one supplementary irrigation during seed planting and flowering, and twice irrigation during flowering and seed set stage increased the yield by 3%, 14%, and 48%, respectively, compared to rainfed conditions. Taken together, the results indicated that to achieve a reasonable economic performance in the studied area, at least two supplementary irrigations are needed in safflower production systems.

**Keywords:** carotenes, chemical fertilizer, chlorophyll, critical period of irrigation, xanthophylls

### Introduction

The effects of climate change during the recent decades have been more evident in semi-arid regions. For example, the occurrence of dry spells at the end of the spring season has become more prevalent [19]. The decrease in annual precipitation to below the threshold of crop production in these areas has made the performance of rainfed crops (without irrigation) not economical and affordable in some years [1]. The safflower plant is one of the annual crops of

the Asteraceae family, which is suitable for semi-arid areas due to its special root structure and depth of root distribution in the soil profile. This multipurpose plant is used for fodder, poultry feeding, oil extraction with industrial applications, and dyeing [3]. However, the lack of sufficient information and technical instructions for the cultivation of this plant has placed it among the underutilized and neglected plants. The production of safflower during 2022 in Iran was 4850 t, which was harvested from 3658 ha. Kazakhstan with about 447,500 tons of safflower seeds accounts for about 45% of the world's total production, and Russia with about 223,000 tons and a share of 22.3% is in second place in safflower production. India is one of the other important producers of safflower seeds with 57,000 t [4]. Although the statistics show that safflower is considered a minor crop compared to other oilseed products, due to its special agricultural characteristics and its relatively high compatibility with semi-arid conditions, its position should be maintained and promoted in crop rotations.

In semi-arid areas with less than threshold rainfall, it is necessary to use supplementary irrigation as agronomic management for balancing the crop water requirements. Supplementary irrigation is an efficient method that includes the application of a small amount of water from underground water sources or harvested runoff under rainfed conditions during the critical growth stage of plants to return to normal growth and achieve acceptable economic yield [8]. However, the key stage of supplemental irrigation application can vary according to plant species, climatic, and edaphic conditions. Durable solar radiation, high temperature at the end of growth season, low and unpredictable precipitation, predominant rainfall during the winter season (rosette stage), low soil water storage capacity, high wind speed, High waste of water without physiological function and high potential evapotranspiration are the prominent characteristics of semi-arid areas [12]. The application of supplementary irrigation increased the yield of fresh fodder and phosphorus content in safflower leaves compared to rainfed conditions. However, it decreased the crude protein and potassium content in the shoot [2].

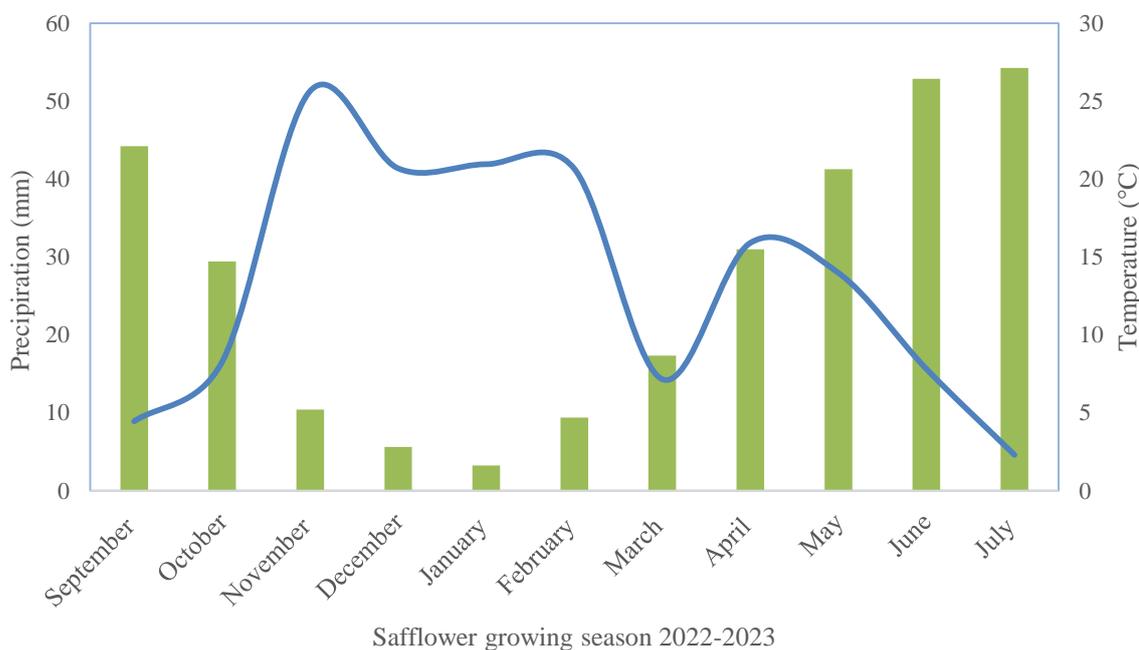
The effect of supplemental irrigation on photosynthetic and protective pigments in safflower has not been well investigated. Our previous studies showed that the use of one-time and two-time supplemental irrigation in chickpea plants in western Iran improved the chlorophyll content by 22% and 29% and increased the seed yield by 30% and 62%, respectively, compared to rainfed conditions [13].

Leaf chlorophyll content decreased under drought stress due to the accumulation of reactive oxygen species, and increased structure decomposition and decreased biosynthesis of pigments. Among the photosynthetic pigments, carotenes and xanthophylls play a role as protectors of the photosynthetic system and one of the signaling components in stress conditions, as well as in the scavenging of the reactive oxygen species [14]. However, due to the severe nutrient deficiency in the soils of semi-arid regions, it seems that in addition to supplying the required moisture, special attention should be paid to the supply of nutrients needed by plants [21]. The previous findings showed that water supply during the flowering and pollination stage is very vital for safflower and nitrogen supply from chemical and organic fertilizer sources with ratios of 50:50 and 75:25 had the best effect on improving safflower growth [18]. The use of combined chemical and biological fertilizers in rainfed chickpea fields improved the yield

significantly [20]. Supplying the water and nutrients improved the physical properties of safflower seeds such as seed diameter, surface area, bulk density, and sphericity. The best characteristics were obtained by providing moisture in the reproductive stage and the combined application of chemical and organic fertilizers [6]. However, the long growth cycle in winter safflower and the lack of financial support regarding the guaranteed purchase of products are other reasons for the low production rate in semi-arid regions. The purpose of the present experiment was to investigate different levels of supplemental irrigation under rainfed conditions and the use of chemical fertilizers and farmyard manure on photosynthetic pigments and components of safflower in the semi-arid region in the northwest of Iran.

### Materials and Methods

The present experiment was carried out in the semi-arid and elevated region of Hashtroud in northwestern Iran and on the northeastern slopes of Sahand Mountain (with latitude and longitude 46°54'E, 37°32'N, 1800 meters above sea level) during the 2022-2023 crop seasons. In terms of climate, the region was one of the semi-arid-cold regions with predominant winter rainfall, and the amount of rainfall during the growing season (September-July) was estimated at 294 mm. The curve of temperature and precipitation in the studied area is shown in Figure 1.



**Fig. 1: Precipitation and monthly temperature in Hashtroud region-northwest of Iran during the safflower growth season of 2022-2023.** Data is taken from Hashtroud meteorological station.

The texture of the investigated farm soil was clay loam and its chemical properties were pH: 7.41, organic matter content: 0.72%, total nitrogen percentage: 0.113%, electrical conductivity: 0.83 dS m<sup>-1</sup>, available phosphorus: 12.14 ppm, potassium: 294 ppm, iron: 1.62 ppm, zinc: 3.27 ppm.

The experiment was conducted as a factorial split-plot (3×5) based on an arbitrary complete block design with three replications. The main plots were allocated to different irrigation regimes (I<sub>1</sub>: rainfed or no-irrigated condition, I<sub>2</sub>: one-time irrigation after seeds

planting, I<sub>3</sub>: supplementary irrigation in the flowering stage, I<sub>4</sub>: supplementary irrigation in the flowering and seed set stages, I<sub>5</sub>: well-irrigated conditions). Between the main plots, two meters of margin were considered to prevent moisture leakage. Different levels of fertilizer were used in sub-plots. Fertilizer treatments were C: control or non-use of fertilizers, NPK: chemical nitrogen, phosphorus, and potassium, and FYM: utilization of 20 t ha<sup>-1</sup> farmyard manure.

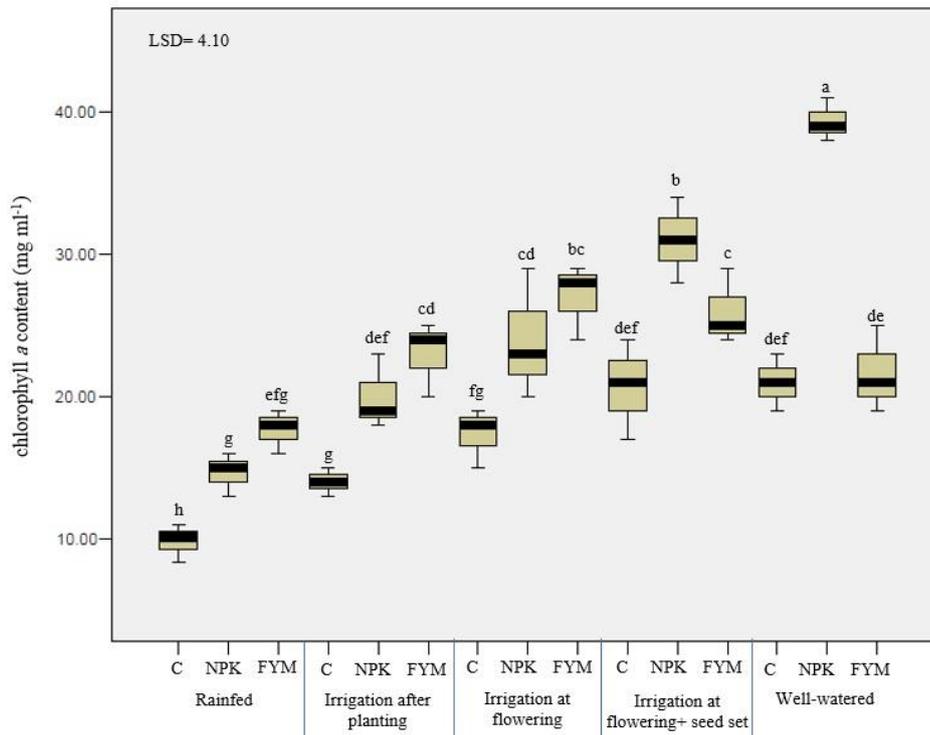
NPK consumption was done based on the results of regional soil analysis and the recommendations of extension organizations. During planting, 80 kg ha<sup>-1</sup> of phosphate was consumed through triple superphosphate fertilizer, 40 kg ha<sup>-1</sup> of potash through potassium sulfate, and 40 kg ha<sup>-1</sup> of nitrogen through urea fertilizer. 40 kg ha<sup>-1</sup> of nitrogen was used during the stem elongation stage (BBCH= 21) and also at the appearance of the main heads (BBCH= 50) [7]. Before the seed sowing on 20 September 2022, the field was plowed, using a reversible moldboard plow and a two-way disc. The main plots were divided in the third decade of September and fertilizers were applied simultaneously with planting. Completely decomposed manure was obtained from calf farming. 20 t ha<sup>-1</sup> of rotted farmyard manure was integrated with the topsoil by a disk and a rotary harrow. The applied farmyard manure contained 31.1% organic matter, pH: 7.56, electrical conductivity: 3.61 ds m<sup>-1</sup>. The percentage of essential elements in FYM was 1.98% N, 0.92% P, 2.03% K, 1.23% Ca. Each experimental unit was 4×3 meters and included 7 planting rows with a distance of 60 cm between the rows. Intra-row spacing was 10 cm. The seeds of the Safeh variety, which is relatively resistant to drought stress and heat at the end of the season, were obtained from the Pakan Seed Company of Isfahan and were planted manually on the top of the ridge in a single row on October 1. After planting the seeds, the main plots of I<sub>5</sub>, I<sub>4</sub>, I<sub>3</sub>, and I<sub>2</sub> were surface irrigated (100 mm) using polyethylene pipes equipped with water volume measuring meters. During vegetative growth (autumn season), weeds were controlled once by hand. Weed control was repeated two times during the stem elongation and growth of lateral branches. Implementation of I<sub>3</sub> irrigation was done during 50% flowering of the field (BBCH= 65) and I<sub>4</sub> plots were irrigated at flowering (BBCH= 65) and at the beginning of the seed set stage (BBCH= 70). The depth of irrigation was 110 mm in each of the mentioned stages. Canopy spread was obtained in the seed stage by measuring the diameter of the canopy using a meter. The total amount of water used through irrigation in well-irrigated conditions was 540 mm.

In the middle of the seed set stage, 5 grams of upper-developed leaves were cut and collected in the experimental plots to extract photosynthetic pigments. After placing the leaves in ice flux and transferring them to the laboratory, chlorophyll *a* and *b* and carotenes were extracted by the method of Lichtenthaler et al (1987) with 50 ml of 80% ethanol [17]. Centrifugation was performed at room temperature for 3-5 minutes at a speed of 500 g until the solution became completely clear. Then, the absorbance of 661.6, 644.8, and 470 nm wavelengths by the leaf extract was checked in a spectrophotometer (Beckman DU 520, USA). Pigmentation measurement was calculated according to Golkar et al. (2009) equations [9]. High performance liquid chromatography (HPLC) methods described by Pasaporte et al. (2014) was used for assessment of xanthophylls content. The HPLC (Shimadzu, Japan) was equipped with C18 column (150 mmL. × 4.6 mmI.D., 4 μm) and UV-VIS detector (450 nm).

In the physiological maturity stage (browning of the heads) using quadrats, plants of 1m<sup>2</sup> were randomly harvested in experimental units and after measuring the height of the plant and counting the heads, they were dried in an oven at a temperature of 70 °C, and aboveground biomass was calculated with a scale. After grinding and separating the seeds, the yield per surface unit was calculated. Variance analysis of the data for the evaluated traits was performed with SAS software. The comparison of the means was executed using the LSD test at the 5% level. Principal component analysis was carried out using Minitab software. Figures and box plots were drawn with Excel and SPSS software.

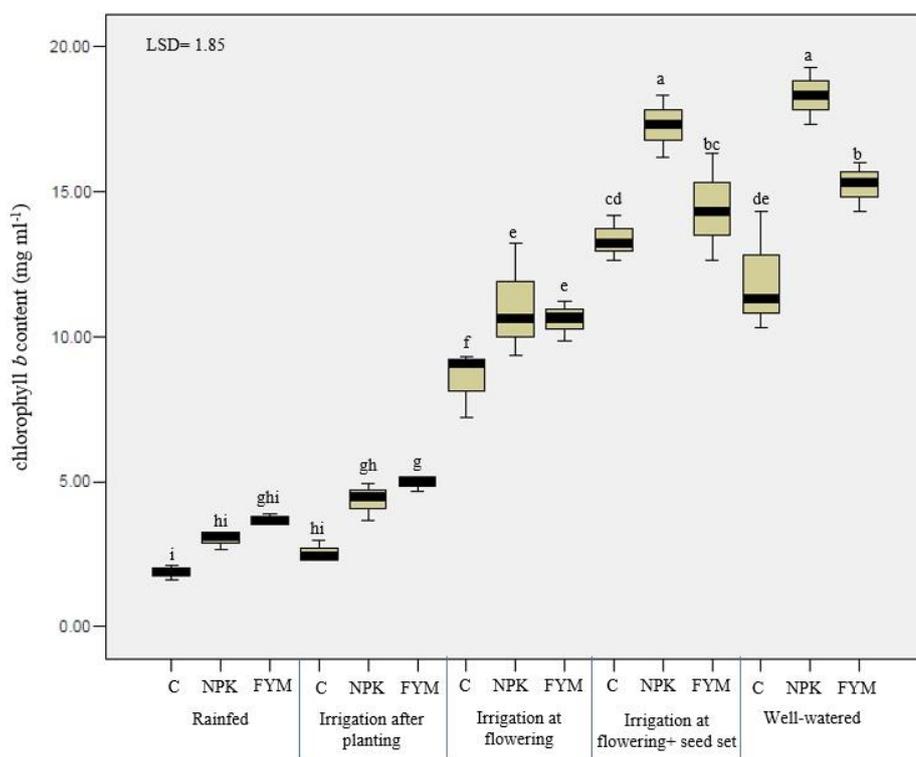
## Results

Evaluation of chlorophyll *a* showed that in addition to the main effects of irrigation (I) and fertilizer use (F), the mutual effect of I×F was significant at the 1% level. The highest amount of chlorophyll *a* was recorded in the conditions of full irrigation with the use of chemical fertilizers (39.33 mg.ml<sup>-1</sup>) and the lowest amount was recorded in the plants grown in rainfed conditions without the use of fertilizers (9.82 mg.ml<sup>-1</sup>). Under well-irrigated conditions (I<sub>5</sub>) and two-times supplementary irrigation (I<sub>4</sub>), the application of chemical fertilizers had a greater effect on chlorophyll *a* compared to the application of FYM. I<sub>5</sub> improved the content of this pigment by 94% compared to rainfed conditions (Figure 2).



**Fig. 2: Mean comparison of chlorophyll *a* content in safflower leaves grown under different conditions of supplementary irrigation and application of animal and chemical fertilizers in the Hashtroud region in the northwest of Iran.** The middle line of the boxes shows the average. Boxes with similar letters do not have statistically significant differences ( $p < 0.05$ ). The vertical lines in each box indicate the dispersion of the collected data. Each data was measured with at least three repetitions.

The interaction effects of I×F on chlorophyll *b* concentration were statistically significant at the 1% level. The plants grown under the two supplementary irrigation and well-watered conditions along with the application of NPK showed the highest concentration of chlorophyll *b* with 18.31 and 17.28 mg.ml<sup>-1</sup>, respectively. The difference in chlorophyll *b* among fertilization levels under well-watered conditions was evident. The lowest amount of chlorophyll *b* was recorded under water deficit conditions (I<sub>1</sub> and I<sub>2</sub>) without fertilizer application, with values of 1.86 and 2.59, respectively. Chlorophyll *b* content in plants grown under I<sub>1</sub>, I<sub>2</sub>, I<sub>3</sub>, and I<sub>4</sub> was 81%, 74%, 34%, and 1% lower compared to well-irrigated conditions. The use of NPK fertilizers and FYM increased chlorophyll B by 55% and 39% compared to control (Figure 3).

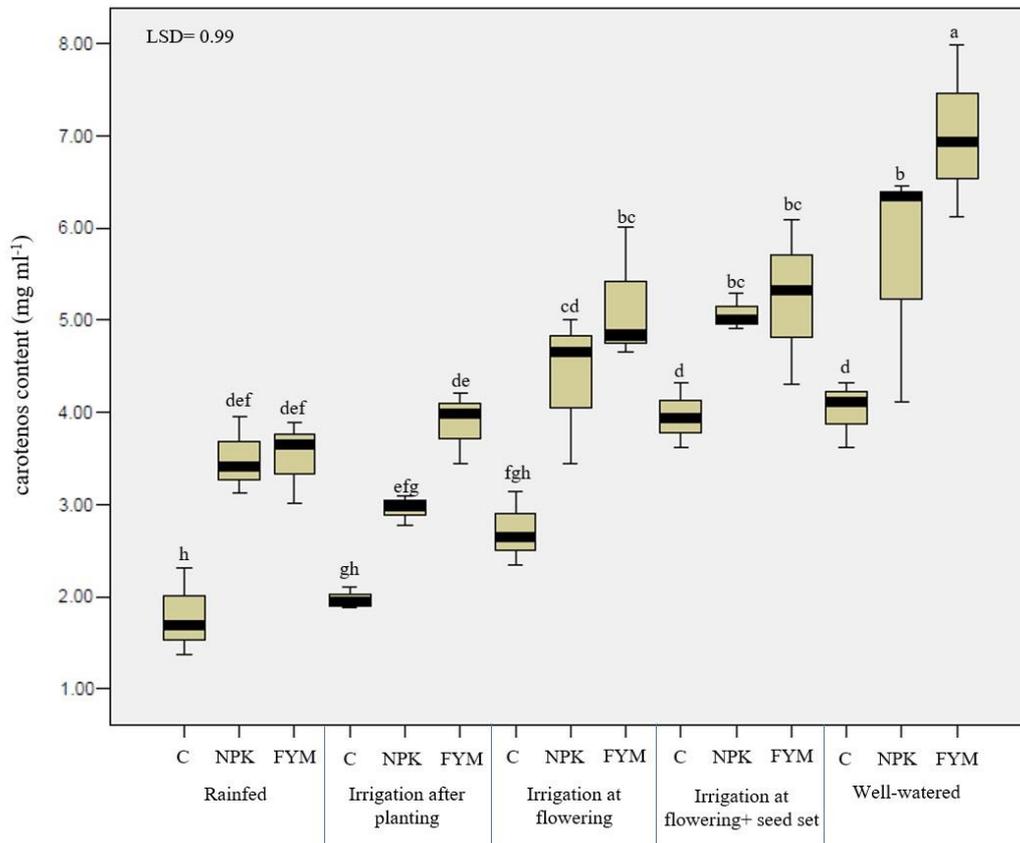


**Fig. 3: Assessment of chlorophyll *b* content in safflower under different soil moisture regimes and various fertilization conditions in the semi-arid region of Hashtroud-Iran.**

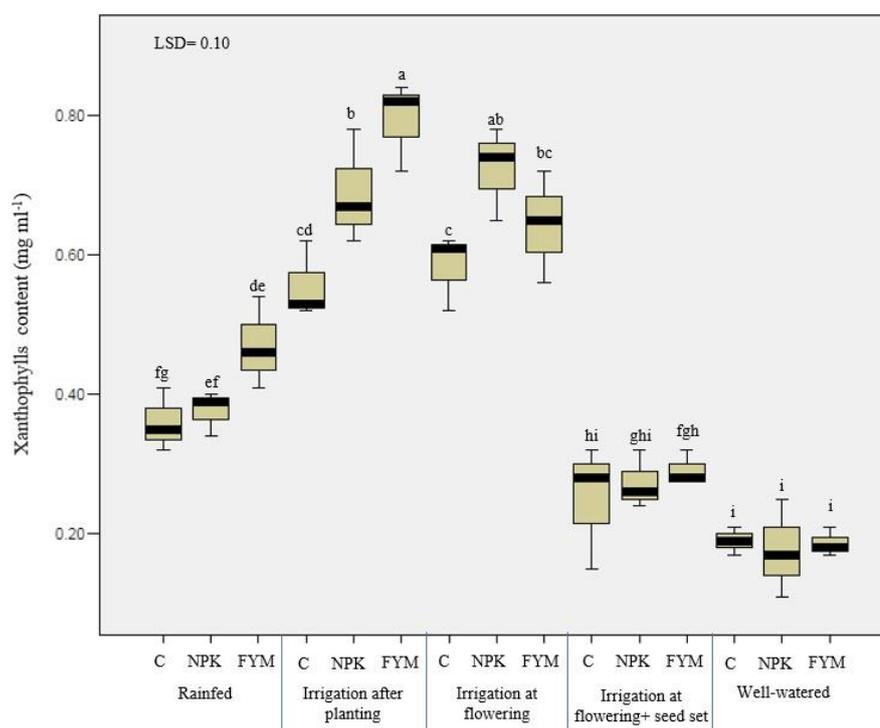
Assessment of carotenes content in the leaves showed that the main effects of irrigation and fertilizer on this parameter were statistically significant at a 1% level. The highest carotenes content was recorded under well-irrigated conditions (5.55 mg.ml<sup>-1</sup>). The carotenes content of plants grown under I<sub>4</sub>, I<sub>3</sub>, I<sub>2</sub>, and I<sub>1</sub> conditions was 14%, 26%, 47%, and 45% lower than well-irrigated conditions, respectively. Means comparison of carotenes content in fertilizer levels showed that the use of FYM and NPK increased the amount of this pigment by 71% and 49% compared to the control. The highest carotenes content was observed under the condition of well-irrigated+ FM (7.013 mg.ml<sup>-1</sup>) and rainfed+ without fertilizer application (1.79 mg.ml<sup>-1</sup>). Under supplemental irrigation conditions (I<sub>3</sub> and I<sub>4</sub>), the application of FYM had a greater increasing

effect on carotenes content compared to NPK. Application of FYM under I<sub>3</sub> and I<sub>4</sub> conditions compared to NPK application improved carotenes content by 5% and 18% (Figure 4).

The main effects of irrigation and fertilizer application had a significant effect on the xanthophylls content at a statistical level of 1%. The interaction effects of I×F on this pigment were also significant. The mean comparison of combined treatments showed that the highest amount of xanthophylls was recorded under I<sub>2</sub>+ FYM (0.792) and I<sub>3</sub>+ NPK (0.72) conditions. The lowest amount of xanthophylls was observed in plants grown under well-irrigated conditions (regardless of the type of fertilizer used). The results showed that increasing water supply decreased the amount of this pigment. The content of xanthophylls increased significantly under rainfed conditions and supplemental irrigation with the application of FYM. Applying two and one-time supplemental irrigation improved the xanthophylls content by 70% and 62%, respectively, compared to rainfed conditions (Figure 5).



**Fig. 4: Evaluation of the content of carotenes in safflower leaves grown at different levels of irrigation and application of chemical and organic fertilizers in the semi-arid region of Hashtroud in the northwest of Iran.**

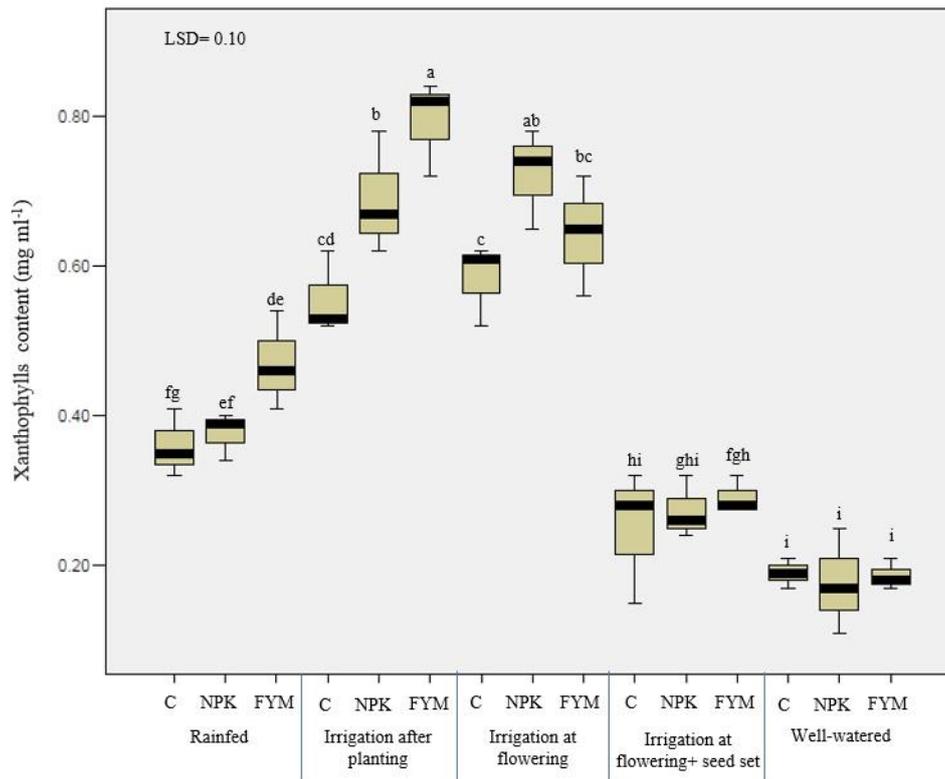


**Fig. 5:** The effect of application of NPK fertilizers and farmyard manure under different irrigation conditions on the xanthophylls pigment content in safflower leaves grown in the semi-arid region of Hashtroud-Iran.

ANOVA results indicated that the interaction effects of I×F for plant height were significant (Table 1). The tallest plants were observed under well-irrigated conditions with the application of NPK (160.78 cm) and by the application of FYM (147 cm). Water supply through irrigation had a significant effect on the plant height, so one irrigation after seed planting increased the plant height by 5% compared to rainfed conditions (Table 1). Under the rainfed and supplemental irrigation conditions not using the fertilizer resulted in the shortest plants.

The measurement of the canopy spread (CS) showed that the application of NPK under well-irrigated conditions produced the largest CS (68.66 cm). However, under I<sub>4</sub> conditions the use of FYM had a greater positive effect on CS compared to NPK. The lowest CS was recorded under rainfed conditions (32 cm) and under I<sub>2</sub> (36 cm) without fertilizer application.

The main effects of irrigation and fertilizer on capitulum diameter (CD) were significant ( $p \leq 0.01$ ). Application of NPK and FYM fertilizers produced the largest CD with 33 mm and 31.7 mm, respectively. The smallest CD was recorded under the conditions of rainfed without fertilizer application (19 mm) and irrigation after planting without fertilizer application (21.6 mm). One-time supply of irrigation in the planting and flowering stages increased CD by 8% and 30%, respectively, compared to rainfed conditions. Although the application of NPK and FYM fertilizers increased CD by 10% compared to the control conditions, no significant difference was observed between obtained CD under chemical and organic fertilizers application. The number of capitula per plant (NCP) was strongly affected by irrigation and fertilizer treatments. Irrigation treatments I<sub>2</sub>, I<sub>3</sub>, and I<sub>4</sub> increased NCP by 29%, 78%, and 94%, respectively (Table 1).



**Fig. 5:** The effect of application of NPK fertilizers and farmyard manure under different irrigation conditions on the xanthophylls pigment content in safflower leaves grown in the semi-arid region of Hashtroud-Iran.

Regardless of the type of fertilizer (chemical or organic), the use of fertilizers increased NCP by 8% compared to the control condition. The number of seeds in the capitulum (NSP) was affected by the mutual effects of  $I \times F$  ( $p \leq 0.01$ ). The highest NSP was achieved with the application of NPK under well-irrigated conditions (27.33). The lowest NSP was obtained under the rainfed condition without soil fertilization (15.46) or with the application of NPK (15.76). Although the use of fertilizers increased the SNP by 8% compared to the control, no significant difference was observed between the fertilizers. Two times supplemental irrigation increased SNP by 42% over the rainfed conditions. Seed weight was affected by irrigation treatments and the lightest seeds were obtained under  $I_4$  irrigation (25.46 g). Grain weight shows slight variations under different conditions. Interestingly, the heaviest seeds were obtained under the conditions of no fertilizer use, and the use of FYM and NPK decreased the seed weight by 1.7% and 3.4%. Seed yield (SEY) was affected by fertilizers. Regardless of the type of fertilizers, soil fertilization increased SEY by 6% over the control. However, the effect of irrigation treatments on SEY was more intense than the effect of fertilizer. Irrigation treatments  $I_2$ ,  $I_3$ ,  $I_4$ , and  $I_5$  respectively increased SEY by 4%, 15%, 48%, and 79% compared to rainfed conditions. The interaction effect of  $I \times F$  for above-ground biomass (AGB) was significant. The highest AGB was obtained under well-irrigated conditions with the use of FYM (5999 kg ha<sup>-1</sup>) or NPK (5973 kg ha<sup>-1</sup>). The lowest AGB was recorded under rainfed conditions without fertilizer application. Plants grown under two times supplemental irrigations, significantly increased their AGB with the application of NPK. Under  $I_3$  irrigation conditions, the effect of FYM application on AGB was more effective than NPK (Table 1).

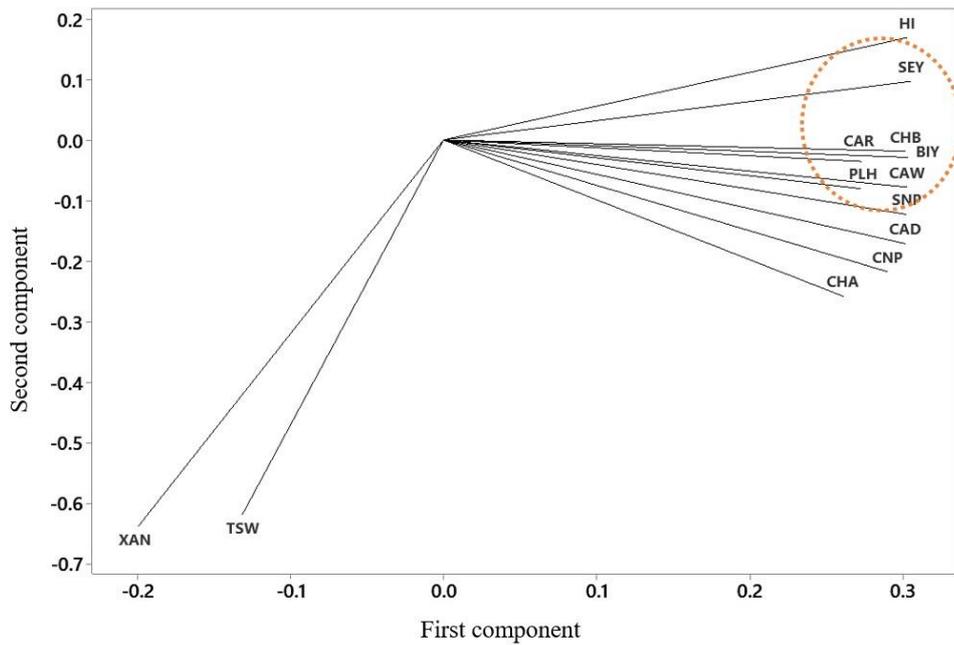
**Table 1: Effect of different supplemental irrigation regimes and fertilizers application on seed yield component and growth parameters of safflower grown in Northwest Iran, Hashtroud.**

Irrigations (I)	PLH	CAW	CAD	CNP	SNP	TSW	SEY	BIY	HI
I <sub>1</sub>	104.98 <sup>d</sup>	35.55 <sup>e</sup>	21.00 <sup>e</sup>	11.84 <sup>e</sup>	15.75 <sup>e</sup>	25.98 <sup>c</sup>	791.00 <sup>e</sup>	4621.33 <sup>e</sup>	17.11 <sup>d</sup>
I <sub>2</sub>	111.73 <sup>c</sup>	40.55 <sup>d</sup>	22.77 <sup>d</sup>	15.39 <sup>d</sup>	18.22 <sup>d</sup>	26.86 <sup>a</sup>	822.56 <sup>d</sup>	4845.16 <sup>d</sup>	16.98 <sup>d</sup>
I <sub>3</sub>	106.36 <sup>d</sup>	43.77 <sup>c</sup>	27.33 <sup>c</sup>	21.14 <sup>c</sup>	19.91 <sup>c</sup>	26.50 <sup>ab</sup>	905.78 <sup>c</sup>	5000.53 <sup>c</sup>	18.11 <sup>c</sup>
I <sub>4</sub>	117.52 <sup>b</sup>	52.77 <sup>b</sup>	29.00 <sup>b</sup>	23.05 <sup>b</sup>	22.46 <sup>b</sup>	25.46 <sup>c</sup>	1172.78 <sup>b</sup>	5441.60 <sup>b</sup>	21.55 <sup>b</sup>
I <sub>5</sub>	149.07 <sup>a</sup>	58.88 <sup>a</sup>	31.58 <sup>a</sup>	26.64 <sup>a</sup>	25.25 <sup>a</sup>	26.24 <sup>b</sup>	1416.33 <sup>a</sup>	5938.84 <sup>a</sup>	23.85 <sup>a</sup>
Fertilizers (F)									
C	107.71 <sup>b</sup>	40.33 <sup>b</sup>	24.73 <sup>b</sup>	18.25 <sup>b</sup>	19.15 <sup>b</sup>	26.57 <sup>a</sup>	985.26 <sup>b</sup>	5094.51 <sup>c</sup>	19.12 <sup>b</sup>
NPK	122.92 <sup>a</sup>	49.55 <sup>a</sup>	27.20 <sup>a</sup>	19.77 <sup>a</sup>	20.81 <sup>a</sup>	25.94 <sup>b</sup>	1046.13 <sup>a</sup>	5174.51 <sup>b</sup>	19.93 <sup>a</sup>
FYM	123.17 <sup>a</sup>	49.06 <sup>a</sup>	27.08 <sup>a</sup>	19.63 <sup>a</sup>	21.00 <sup>a</sup>	26.20 <sup>b</sup>	1033.66 <sup>a</sup>	5239.47 <sup>a</sup>	19.51 <sup>ab</sup>
I <sub>1</sub> C	93.00 <sup>j</sup>	32.00 <sup>h</sup>	19.00 <sup>h</sup>	10.21 <sup>j</sup>	15.46 <sup>h</sup>	26.23 <sup>bc</sup>	772.33 <sup>l</sup>	4520.80 <sup>f</sup>	17.08 <sup>g</sup>
I <sub>1</sub> NPK	109.12 <sup>gh</sup>	36.33 <sup>g</sup>	21.00 <sup>gh</sup>	12.79 <sup>ij</sup>	15.76 <sup>h</sup>	25.70 <sup>cd</sup>	789.00 <sup>hi</sup>	4592.27 <sup>i</sup>	17.18 <sup>g</sup>
I <sub>1</sub> FYM	112.84 <sup>efg</sup>	38.33 <sup>g</sup>	23.00 <sup>fg</sup>	13.53 <sup>hi</sup>	16.04 <sup>gh</sup>	26.03 <sup>bcd</sup>	811.67 <sup>hi</sup>	4750.93 <sup>h</sup>	17.08 <sup>g</sup>
I <sub>2</sub> C	100.02 <sup>ij</sup>	36.00 <sup>gh</sup>	20.66 <sup>gh</sup>	14.70 <sup>sh</sup>	17.70 <sup>fg</sup>	26.91 <sup>ab</sup>	812.33 <sup>hi</sup>	4712.00 <sup>h</sup>	17.24 <sup>fg</sup>
I <sub>2</sub> NPK	115.73 <sup>ef</sup>	43.00 <sup>f</sup>	23.00 <sup>fg</sup>	15.39 <sup>g</sup>	18.33 <sup>ef</sup>	26.80 <sup>ab</sup>	831.67 <sup>h</sup>	4877.33 <sup>g</sup>	17.05 <sup>g</sup>
I <sub>2</sub> FYM	119.45 <sup>de</sup>	42.66 <sup>f</sup>	24.66 <sup>ef</sup>	16.09 <sup>g</sup>	18.63 <sup>ef</sup>	26.86 <sup>ab</sup>	823.67 <sup>h</sup>	4946.13 <sup>g</sup>	16.65 <sup>g</sup>
I <sub>3</sub> C	102.09 <sup>hi</sup>	37.00 <sup>g</sup>	26.00 <sup>de</sup>	20.55 <sup>f</sup>	18.44 <sup>ef</sup>	27.66 <sup>a</sup>	880.33 <sup>g</sup>	4995.20 <sup>ef</sup>	17.62 <sup>efg</sup>
I <sub>3</sub> NPK	102.92 <sup>hi</sup>	45.33 <sup>ef</sup>	28.00 <sup>cd</sup>	21.63 <sup>def</sup>	20.49 <sup>cd</sup>	26.00 <sup>bcd</sup>	911.33 <sup>fg</sup>	4905.07 <sup>fg</sup>	18.57 <sup>e</sup>
I <sub>3</sub> FYM	114.08 <sup>ef</sup>	49.00 <sup>de</sup>	28.00 <sup>cd</sup>	21.25 <sup>ef</sup>	20.81 <sup>cd</sup>	25.83 <sup>cd</sup>	925.67 <sup>f</sup>	5101.33 <sup>e</sup>	18.14 <sup>ef</sup>
I <sub>4</sub> C	104.98 <sup>hi</sup>	44.00 <sup>f</sup>	28.00 <sup>cd</sup>	22.30 <sup>de</sup>	19.83 <sup>de</sup>	25.70 <sup>cd</sup>	1106.67 <sup>f</sup>	5401.07 <sup>d</sup>	20.49 <sup>d</sup>
I <sub>4</sub> NPK	126.06 <sup>d</sup>	54.33 <sup>c</sup>	31.00 <sup>ab</sup>	23.76 <sup>bc</sup>	22.16 <sup>c</sup>	25.16 <sup>d</sup>	1319.33 <sup>d</sup>	5524.00 <sup>c</sup>	22.08 <sup>c</sup>
I <sub>4</sub> FYM	121.52 <sup>de</sup>	60.00 <sup>b</sup>	28.00 <sup>cd</sup>	23.10 <sup>bcd</sup>	25.40 <sup>b</sup>	25.53 <sup>cd</sup>	1192.33 <sup>d</sup>	5399.73 <sup>d</sup>	22.09 <sup>c</sup>
I <sub>5</sub> C	138.46 <sup>c</sup>	52.66 <sup>cd</sup>	30.00 <sup>bc</sup>	23.50 <sup>bc</sup>	24.33 <sup>b</sup>	26.36 <sup>bc</sup>	1354.67 <sup>c</sup>	5843.47 <sup>b</sup>	23.20 <sup>b</sup>
I <sub>5</sub> NPK	160.78 <sup>a</sup>	68.66 <sup>a</sup>	33.00 <sup>a</sup>	26.26 <sup>a</sup>	27.33 <sup>a</sup>	26.03 <sup>bcd</sup>	1479.33 <sup>a</sup>	5973.87 <sup>a</sup>	24.76 <sup>a</sup>
I <sub>5</sub> FYM	147.97 <sup>b</sup>	55.33 <sup>c</sup>	31.76 <sup>ab</sup>	24.16 <sup>b</sup>	24.13 <sup>b</sup>	26.33 <sup>bc</sup>	1415.00 <sup>b</sup>	5999.20 <sup>a</sup>	23.59 <sup>b</sup>
Significance level									
I	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001
F	<0.0001	<0.0001	<0.0001	0.0006	<0.0001	0.0111	<0.0001	<0.0001	0.0063
I×F	0.0140	<0.0001	0.1487	0.1106	0.0006	0.1518	0.0077	0.0030	0.0938
CV%	4.61	5.54	7.65	5.40	5.27	2.10	7.56	7.29	3.22

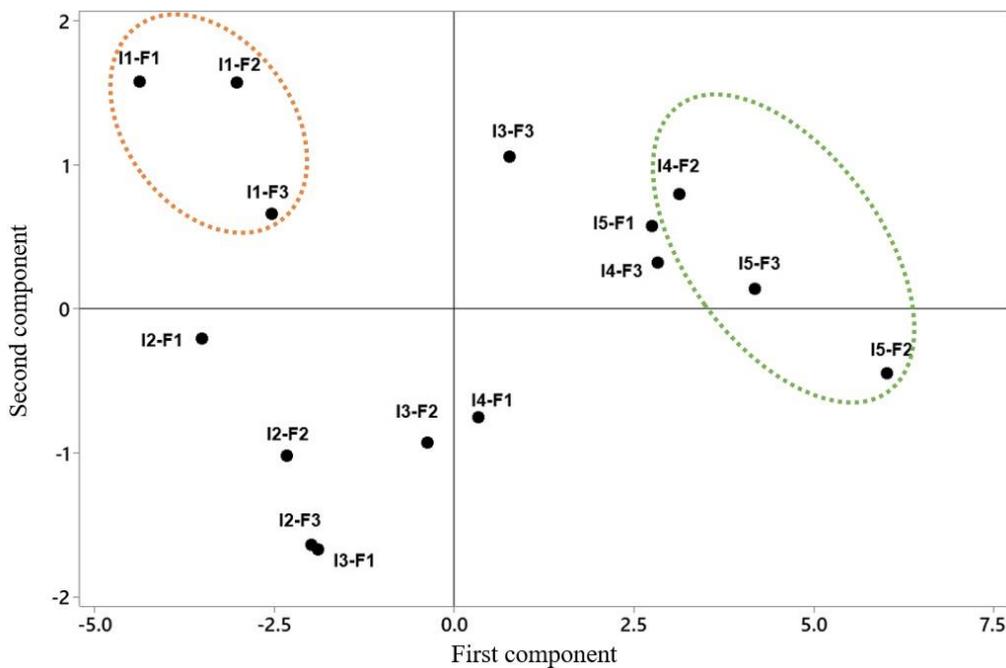
I<sub>1</sub>: rainfed or no-irrigated condition, I<sub>2</sub>: one-time irrigation after seeds planting, I<sub>3</sub>: supplementary irrigation in the flowering stage, I<sub>4</sub>: supplementary irrigation in the flowering and seed set stages, I<sub>5</sub>: well-irrigated conditions, C: control or non-use of fertilizers, NPK: chemical nitrogen, phosphorus, and potassium, FYM: 20 t ha<sup>-1</sup> animal manure. CV: The coefficient of variation (%), PLH: Plant height (cm), CAW: canopy lateral spread (cm), CAD: head or capitulum diameter (mm), CNP: capitulum number per plant, SNP: seed number per capitulum, TSW: thousand seed weight (g), SEY: seed yield (kg ha<sup>-1</sup>), BIY: above-ground biomass (kg ha<sup>-1</sup>), HI: harvest index (%). At the significance level, *p* values less than 0.05 (*p* < 0.05) and 0.01 (*p* < 0.01) are significant at 95% and 99%, respectively. The means with various letters in each trait (column) are statistically different.

The pulled-out plot from the principal component analysis showed that the first component was able to separate different irrigation regimes. The best results were obtained from well-irrigated conditions and two times supplemental irrigation which were distinguished from other water deficit conditions. Also, the second component was able to distinguish the combined effect of fertilizer and water shortage from optimal conditions (Figure 7). The best plant performance was obtained under I<sub>5</sub> treatment with the use of NPK or FYM. The faintest effects of fertilizers were observed under rainfed conditions. Under I<sub>4</sub> treatment, the effectiveness of NPK was more evident than that of organic fertilizers.

The angular correlation between the evaluated traits is shown in Figure 6. Low angles indicated a significant positive correlation between components. Seed yield had a significant and positive correlation with the content of chlorophyll *b*, carotenes, canopy area, number of seeds per plant, ABG, and plant height.



**Fig. 6:** Estimation of the correlation between different agricultural traits, yield components, and photosynthetic pigments in sunflowers grown under different irrigation and fertilization regimes. Smaller angles between traits indicate a positive and significant correlation. PLH: Plant height, CHA: chlorophyll *a* content, CHB: chlorophyll *b* content, CAR: carotenes content, XAN: xanthophylls content, CAW: canopy lateral spread, CAD: head or capitulum diameter, CNP: capitulum number per plant, SNP: seed number per capitulum, TSW: thousand seed weight, SEY: seed yield, BIY: above-ground biomass, HI: harvest index



**Fig. 7:** Principal component analysis (PCA) showing a plot of the first two PCs for all combined treatments (irrigation regimes and fertilizer application) on safflower grown in the Hashtroud region. **I<sub>1</sub>**: rainfed or no-irrigated condition, **I<sub>2</sub>**: one-time irrigation after seeds planting, **I<sub>3</sub>**: supplementary irrigation in the flowering stage, **I<sub>4</sub>**: supplementary irrigation in the flowering and seed set stages, **I<sub>5</sub>**: well-irrigated conditions, **F<sub>1</sub>**: control or non-use of fertilizers, **F<sub>2</sub>**: chemical NPK, **F<sub>3</sub>**: 20 t ha<sup>-1</sup> farmyard manure).

### Discussion

An overview of the soil properties of the investigated area indicated that nutrients deficiencies and organic matter in the soil is one of the important factors limiting the production of safflower. In rainfed condition nutrients can be released as the soil organic matter is decayed by microorganisms. However, due to climatic restrictions, inappropriate crop management and excessive grazing of plant residues the amount of crop residue returned to the soil is very low, therefore the nutrients deficiencies are very prevalent [11]. On the other hand, the evaluation of the amount of precipitation during the growing season showed that the majority of the precipitation falls during the vegetative period or the rosette stage of the safflower. With the initiation of reproductive growth, the amount of rainfall decreased significantly. However, the potential evapotranspiration in the mentioned area significantly increases during the May-August and total evapotranspiration during the development period was about 551 mm [25]. In such a situation, the use of supplemental irrigation is an inevitable management option. Therefore, the performance in rainfed conditions was very low and not economically reliable. It seems that both due to the low amount of precipitation and low soil water storage capacity, the soil moisture level was less than the critical level to produce an acceptable yield under rainfed conditions.

The results showed that the effect of irrigation treatment was much stronger than fertilization. This shows that the restrictions caused by the water shortage in the region have a greater impact compared to the nutritional deficiencies. Water is the most important factor in stimulating cell growth and continuity of cellular processes in plants [5]. In well-irrigated conditions, the use of chemical fertilizers had a greater effect than FYM. This is probably due to the rapid release of essential plant elements by chemical fertilizers, while some positive effects of FM appear in the long term [22]. Water shortage under rainfed conditions also caused a significant decrease in the growth of vegetative organs (canopy width, plant height), a decrease in photosynthetic pigments, and a decrease in the growth of reproductive organs. In another interpretation, water stress affected the source-sink relationships in safflower and significantly reduced both components. It appears that plants grown under water stress and nutrients deficiencies faced with sink and source co-limitation [15]. The significant decrease of chlorophyll *a* and *b* under water shortage conditions was consistent with the findings of Khosrowshahi et al., 2020 [14]. However, the content of xanthophylls showed the highest value under one -time supplemental irrigation in the flowering stage. This may refer to the protective role of this pigment under supplemental irrigation conditions. It seems that under rainfed conditions, due to the prolongation of the period of water shortage and the severe damage to the intracellular processes such as photosynthesis, the plants could not increase their xanthophylls content.

The evaluation of the traits indicated that one irrigation after planting the seeds does not have much improvement effect and it is recommended to use the available water resources during the critical reproductive stage such as of formation of yield components. Considering the significant decrease in the amount of rainfall during reproductive growth as well as the irregular distribution of rainfall during different stages of plant growth, at least two-time supplemental

irrigation seems to be necessary to achieve an acceptable yield. These results confirm the findings of Hazrati et al. (2024) who indicated that the use of supplemental irrigation in the flowering and seed filling stage improved the quantitative aspects of seed yield and oil quality of *Camelina sativa* in the semi-arid areas of northwestern Iran. Although it is thought that drought stress increases seed filling dependency on remobilization in plants [24]. Under rainfed conditions or a one supplemental irrigation at the seed sowing or at the flowering stage was not suitable to stimulate remobilization and compensating the photoassimilates deficiency in the seed filling stage. It seems that the long-term drought conditions can reduce the amount of retranslocation from carbon pool reserves by reducing the rate of photosynthesis and decreasing the storage of nonstructural carbohydrates in stems [15].

### Conclusion

The studied area was faced with a severe water shortage and results revealed that the soil water storage under rainfed conditions alone was not sufficient for the economic production of saffron. Hot and dry spells during the reproductive period had a more reducing effect on yield components. One-time irrigation after seed planting had a negligible effect on the improvement of safflower seed yield. The results showed that to achieve an acceptable yield, it is necessary to apply two supplemental irrigations during the flowering and seed set stages. Among the evaluated fertilizers, the application of FYM under supplemental irrigation conditions had better results. However, the application of NPK was more effective under full-irrigated conditions.

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### EVALUAREA PIGMENTILOR FOTOSINTETICI ȘI A CREȘTERII SPECIEI *CARTHAMUS TINCTORIUS* ÎN DIFERITE CONDIȚII DE UMIDITATE A SOLULUI

#### (Rezumat)

Schimbările climatice, apariția perioadelor de secetă cu precipitații scăzute, precum și gestionarea necorespunzătoare a aplicării îngrășămintelor în zonele semi-aride sunt principalele cauze pentru producția scăzută de șofrănel. Experimentul actual a avut ca scop evaluarea diferitelor regimuri de irigații (I1: irigare pluvială sau neirigat, I2: irigare unică după plantarea semințelor, I3: irigare suplimentară în stadiul de înflorire, I4: irigare suplimentară în stadiile de înflorire și legare a semințelor, I5: irigare suficientă) și utilizarea diferitelor îngrășăminte (C: control fără îngrășăminte, NPK: azot chimic, fosfor și potasiu, FYM: gunoi de grajd 20 t/ha) în regiunea înaltă și semi-aridă din Hashtroud. Rezultatele au arătat că cele mai mari cantități de clorofilă a (39,33 mg/ml) și clorofilă b (18,3 mg/ml) au fost înregistrate în condițiile I5+ NPK, iar cele mai mici au fost înregistrate de plantele cultivate în condițiile I1+ C. Cele mai mari concentrații de xantofile au fost înregistrate la plantele cultivate în condițiile I2+FYM (0,79 mg/ml) și I3+NPK (0,72 mg/ml), iar cea mai mică cantitate a fost în cazul condițiilor I5+NPK (0,17 mg/ml). Cele mai înalte plante (160,78 cm), cea mai extinsă ramificare (68,66 cm), cel mai mare diametru al inflorescenței (33 mm) și cel mai mare număr de inflorescențe per plantă (26,2) au fost înregistrate în condițiile I5+ NPK. Cele mai mari 1000 de semințe au fost observate în condițiile I3+C (27,66 g), iar cele mai ușoare semințe (25,16 g) au fost obținute de la plantele cultivate în condițiile I4+NPK. Utilizarea unei irigații suplimentare în timpul plantării și înfloririi și a irigații de două ori în timpul înfloririi și formării semințelor a crescut randamentul cu 3%, 14% și, respectiv, 48%, comparativ cu condițiile normale de precipitații. Concluzionând, rezultatele au indicat că, pentru a obține o performanță economică rezonabilă în zona studiată, sunt necesare cel puțin două irigații suplimentare în sistemele de producție a șofrănelului.

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