

## Effects of Sowing Date and Irrigation Treatment on Safflower Seed Quality

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### ABSTRACT

Field experiments were conducted to investigate the effects of sowing dates and irrigation on seed quality of a spring-type safflower cultivar, in Karaj-Iran, during 2008 and 2009 growing seasons. A split plot design based on a randomized complete block layout with three replications was used in which sowing dates and irrigation treatments comprised the main plot and sub-plot, respectively. The results showed the highest oil yield, oil content, protein yield, and linoleic acid content of safflower seed for sowing date of 19<sup>th</sup> of April and under non-water stress conditions. The highest seed protein, oleic acid, and palmitic acid contents were obtained for sowing dates of May 5<sup>th</sup> and 20<sup>th</sup> along with ceasing irrigation at heading, flowering, and seed filling stages, respectively. Under arid and semiarid Mediterranean conditions prevailing in Karaj, late sowing date led to a greater simultaneity of reproductive stages and higher temperature, which made the negative effect of water stress more prominent than in the early sowing dates.

**Keywords:** Fatty acid profile, Late sowing date, Seed oil yield, Water stress.

### INTRODUCTION

The deep-root system of safflower (*Carthamus tinctorius* L.) enables the plant to capture moisture and nutrients from the soil depths and tolerate heat and dry conditions when established (Majidi *et al.*, 2011). Safflower oil is composed of unsaturated fatty acids including linoleic acid (18:2) and oleic acid (18:1), and saturated fatty acids as palmitic acid (16:0) and stearic acid (18:0). It is one of the richest sources of linoleic acid among the commercially available oil (Yeilaghi *et al.*, 2012). Safflower seed oil contains about 71–75% linoleic acid, 16–20% oleic acid, 6–8% palmitic acid, and 2–3% stearic acid (Velasco and Fernandez-Martinez, 2001). This high level, combined with an absence

of linolenic fatty acid, which has made safflower oil appealing to consumers, initially as quick-drying oil, more recently, as edible oil with the highest linoleic acid.

Different sowing dates caused flowering and seed development to occur during periods of widely different temperatures, radiation, and day length. Yau (2006) stated that later sowing of spring safflower in semi-arid and high elevation Mediterranean environment resulted in lower seed yield as later flowering does not allow escape from the terminal drought and heat. It is likely that increased temperature and water stress during seed filling was a major cause of reduction of oil content and thus increased protein content due to late sowing (Hocking and Stapper, 2001). Sowing date can be a major factor that affects oil content and fatty

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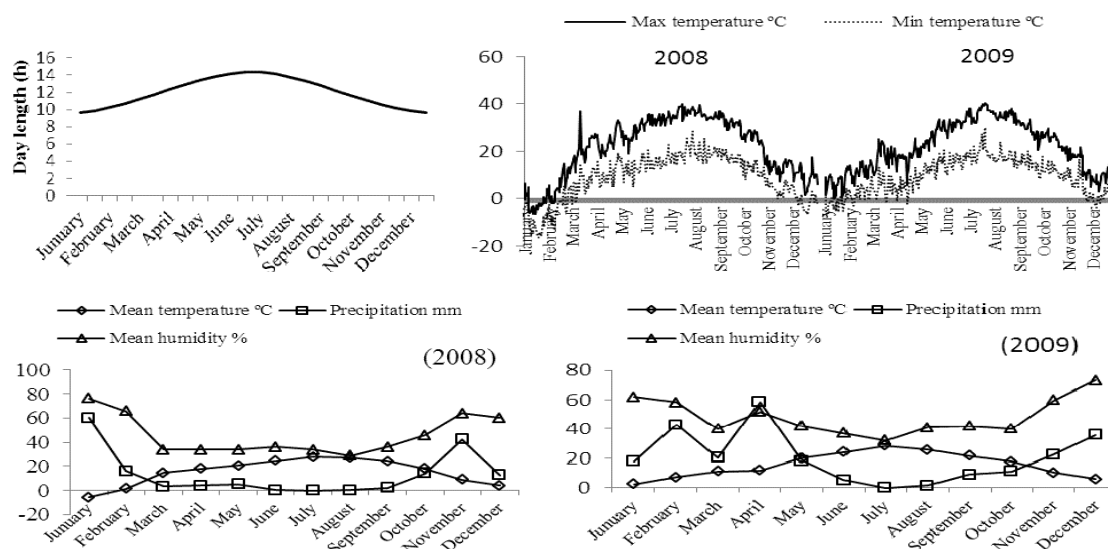


acid composition at the time of seed development (Samanci and O'zkaynak, 2003). The ratio of oleic and linoleic acids in seed oils is to a large extent dependent upon environmental conditions, particularly moisture and temperature, during seed maturation (Gecgel *et al.*, 2007). Water deficit stress usually results in shorter plants, less branching; lower seed yield and oil content lead to lower oil yield. Pasban Eslam (2011) indicated that drought stress during seed filling stage in the spring safflower genotypes significantly decreased seed and oil yields in arid and semi-arid regions. The same author stated that seed yield had a significantly positive (0.97) correlation with oil yield. The availability of water in both vegetative and reproductive stages of crop growth influences the quality, although the environmental factors are more influential in reproductive stages, specifically, the seed filling period. The research by Gecgel *et al.* (2007) revealed that the oil content and the four major fatty acids in safflower seeds were affected by sowing dates, and the availability of water from the flowering to maturity resulted in increase oil content. Ensiye and Khorshid (2010) reported that both oil content and the oil fatty acid composition showed significant differences in relation to the water regime, sowing date and genotype. Regarding the proper irrigation level, it is essential to have both higher yield potential and less water consumption. The purpose of the present study was to research the response of safflower qualitative traits to water deficit during vegetative, flowering, and seed formation stages, with a view to different sowing dates in spring.

## MATERIALS AND METHODS

The study was carried out in an agricultural research station of Karaj in the South-West of Tehran (Lat. 35°, 59' Long. 50°, 75', Elevation: 1,312 m), Iran. Experimental layout had split plot arrangement based on randomized complete block design with three replications

during two growing seasons (2008 and 2009). The treatments comprised three sowing dates (19 April, 5 and 20 May) as main plots; and four irrigation treatments ( $I_0$ : Irrigation after every 60mm evaporation from class "A" pan, as control;  $I_1$ : Ceasing irrigation at heading stage;  $I_2$ : Ceasing irrigation at flowering stage,  $I_3$ : Ceasing irrigation at seed filling stage) as subplots. All treatments received equal amounts of water in each irrigation, which was measured with the help of a 15 cm throat Parshall flume fixed in the irrigation channel. Day length (h), temperature (°C), relative humidity (%) and precipitation (mm) were obtained from Karaj meteorological substation (Figure 1). Karaj is located in the central regions of Iran and has a mean annual temperature of 14.1°C and an average rainfall of 251 mm. Soil samples were taken using augers from 0-30 cm before sowing. Soil was silty loam in texture with pH 7.4, 0.53% organic matter; total nitrogen of 0.11 ppm, available phosphorus of 6.3 ppm, exchangeable potassium of 275 ppm, with no salinity problems ( $EC = 2.36 \text{ dS m}^{-1}$ ). On the basis of soil analysis, 120 kg ha<sup>-1</sup> urea and 80 kg ha<sup>-1</sup> ammonium phosphate were applied to the site and harrowed before seedbed preparation. Nitrogen was applied in two applications: half before sowing and the remaining half was top dressed at flower-bud-visibility stage. Each plot was 10 m<sup>2</sup> consisting of eight rows, 5 m long and 25 cm apart. Seeds were sown 4 cm apart at about 4-5 cm depth. A 1.0 m alley was left around each plot. Plots were over seeded and subsequently thinned to final density of about 100 plants m<sup>-2</sup> at seedling stage. Weeds were controlled by both Trifluralin (2.5 L ha<sup>-1</sup>) as pre emergence and by hand as needed. At maturity, the six central rows of each plot were harvested for seed yield determination. Subsamples were dried at 75°C for moisture determination. Seed yield was adjusted to 9% moisture content and all other measurements were reported on a dry weight basis. Twenty plants were randomly collected from the central six rows with edging shears (0.1 m cutting width) and the following characteristics were recorded for yield (kg ha<sup>-1</sup>) and % content of oil, protein, linoleic, oleic,



**Figure 1.** Ambient temperature, rainfall, and relative humidity during safflower growing season of 2008 and 2009 in Karaj.

stearic and palmitic acids. Oil content was determined using nuclear magnetic resonance (Jambunathan *et al.*, 1985). To estimate protein content, nitrogen concentration was determined using a Technicon autoanalyzer (Singh and Jambunathan, 1980). A factor of 5.30 was used to convert nitrogen into crude protein content (FAO, 1970). Seed samples were taken for total fatty acid analyses. Total fatty acid content was analyzed using a method modified by Arslan (2007). In this method, seed samples were soaked in 2 mL of 2% sulphuric acid in dry methanol for 16 hr, at lab temperature followed by 80 minutes of heating at 90°C to convert the fatty acids into methyl derivatives (FAMES). Methyl heptadecanoate (17:0-ME) was added as an internal standard. The FAMES were extracted in 2 mL water and 3 mL hexane and then determined by gas liquid chromatography (GLC). The fatty acid methyl ester composition was analyzed by using a Varian 3400 gas chromatography equipped with a Supelcovax-10 fused silica capillary column (30 m×0.25 µm film thickness). The column's initial temperature was kept at 160°C for 15 minutes; in this temperature an increase could develop at the rate of 5 °C min<sup>-1</sup>. The temperatures of the injector and the detector

(FID) were 240 and 280°C, respectively. The carrier gas was nitrogen with a flow rate of 1-2 mL min<sup>-1</sup>. Split ratio was adjusted to 30 mL min<sup>-1</sup>. The injected volume of the sample was 1 µL. Fatty acids were identified by retention time relative to that of an authentic standard. The FAMES were identified by comparing the retention times with those of the standards. Fatty acid content was computed as weight percentage of the total fatty acids by using the GC area counts for various FAMES.

### Statistical Analysis

Analysis of variances of the data for each attribute and combined analysis of the split plot designs in two years were computed using the SAS computer program (SAS, 2001). F test in combined analysis of the experiments was carried out using expected values of the mean squares in which year and replication were assigned as random variables and sowing dates and irrigation treatments were considered as fixed variables. The Fisher's least significant difference (LSD) test at 5% level of probability was used to test significant effects. The MSTAT-C software package was used to test significant interaction effects



between treatments (MSTAT, 1993). The coefficient of linear regression equation between the traits mean, as the dependent variables, and degree day (D.D.) throughout the growth season of safflower, as the independent variable, were calculated by SAS. Accumulated growth degree days (GDD) were calculated by summing the daily degree day values ( $^{\circ}\text{C}$ ) obtained by adding the maximum and minimum temperatures for the day, dividing by two and subtracting the base temperature, which, for safflower, was taken as  $5^{\circ}\text{C}$  (Table 1).

## RESULTS

### Interaction Effect of Year with Sowing Dates

The interactive effect of year with sowing date was significant for all studied parameters, except for protein yield (Table 2). The values of safflower oil yield, oil content and linoleic acid content were significantly higher for the first sowing date in 2009 compared to 2008, while more protein, oleic, stearic and palmitic acid contents were recorded with second sowing date in 2008 than in 2009 (Figure 2).

### Effect of Irrigation Treatments

Differences between irrigation treatments were significant for all studied traits, except oleic and palmitic acids content in the

combined analysis of variances (Table 2). The highest oil and linoleic acid contents were obtained from control irrigation treatment, while ceasing irrigation at heading stage resulted in the lowest amount (Table 3). Maximum protein and stearic acid contents were recorded for ceasing irrigation at heading and flowering stages, respectively.

### Interaction Effect of Sowing Dates with Irrigation Treatments

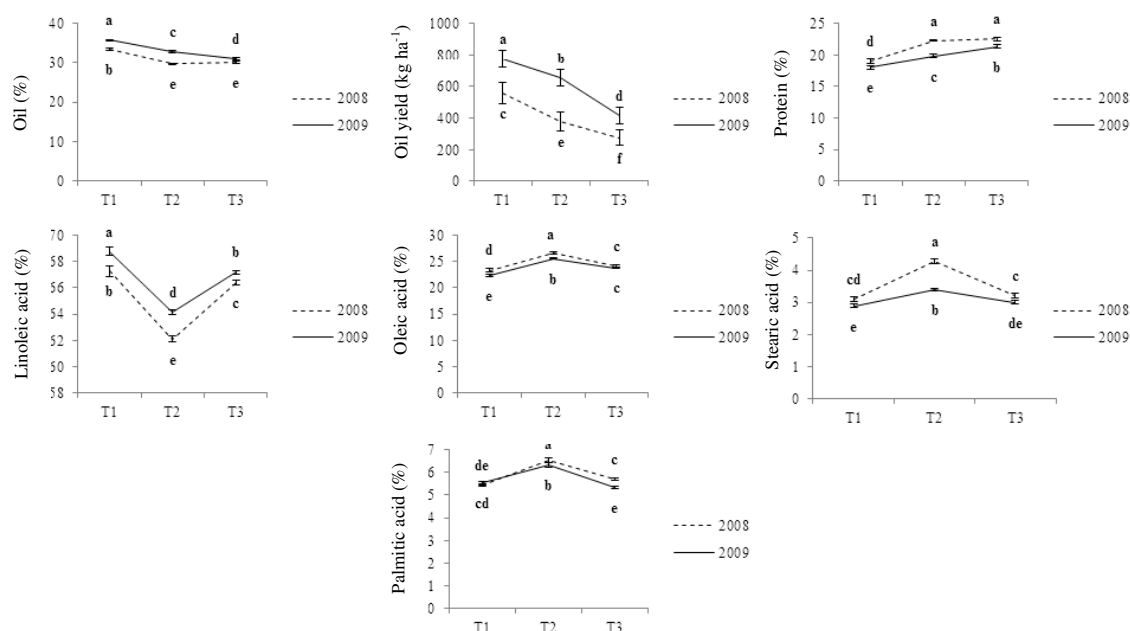
Interaction between sowing dates and irrigation treatments was not significant for safflower growth parameters, except for oleic acid content (Table 2). In the May 5<sup>th</sup> sowing date, all irrigation treatments produced the highest oleic acid content, whereas the lowest values for this trait was recorded for April 19<sup>th</sup> sowing date under normal irrigation and ceasing irrigation at seed filling stage (Figure 3).

### Interaction Effect of Year with Irrigation Treatments

According to the results of the combined analysis of variances for quality components, year-irrigation levels interaction were significant for oil and protein yield and palmitic acid content, while oil, protein, linoleic acid, oleic acid and stearic acid contents were not significant (Table 2). The mean comparison of interaction effect of year with irrigation

**Table 1.** Safflower growing period (Duration) under different treatments of sowing dates and irrigation treatments stress in 2008 and 2009.

		T1				T2				T3			
		I0	I1	I2	I3	I0	I1	I2	I3	I0	I1	I2	I3
2008	Duration (Day)	119	66	85	110	103	59	71	92	81	51	67	73
	GDD ( $^{\circ}\text{C}$ day)	2090	1059	1413	1919	1851	986	1213	1637	1506	902	1219	1348
2009	Duration (Day)	132	91	113	125	124	78	103	113	95	66	69	85
	GDD ( $^{\circ}\text{C}$ day)	2233	1458	1885	2108	2227	1375	1845	2030	1774	1230	1286	1587



**Figure 2.** Interaction effects of year with sowing date ( $\pm$ SE) on oil content, oil yield, protein content and linoleic, oleic, stearic, palmitic acids contents of safflower. Values followed by the same letter or letters are not significantly different at the 5 % level LSD (Fisher's least significant difference test). T<sub>1</sub>: April 19<sup>th</sup>; T<sub>2</sub>: May 5<sup>th</sup>; T<sub>3</sub>: May 20<sup>th</sup>.

**Table 2.** Combined analysis of variance for the effects of year, sowing dates and irrigation treatments on qualitative characters of spring safflower (r= 3).

SOV	df	Oil (%)	Oil yield (kg ha <sup>-1</sup> )	Protein (%)	Protein yield (kg ha <sup>-1</sup> )	Linoleic acid (%)	Oleic acid (%)	Stearic acid (%)	Palmitic acid (%)
Y	1	79.38**	793800**	44.95**	135894**	37.7**	12.77**	3.52**	0.482**
R/Y	4	0.122	183	0.079	136.7	0.17	0.051	0.019	0.007
T	2	114 <sup>ns</sup>	617317*	74.66*	83168*	156.8*	66.2*	4.93 <sup>ns</sup>	7.04*
Y×T	2	6.87**	27100**	3.52**	1931 <sup>ns</sup>	2.61**	1.03*	0.9**	0.283*
R×T(Y)	8	0.241	313.3	0.281	630	0.222	0.15	0.031	0.041
I	3	10.86**	791778**	11.35**	239632**	6.03*	2.06 <sup>ns</sup>	0.422**	0.532 <sup>ns</sup>
T×I	6	0.216 <sup>ns</sup>	5377.4 <sup>ns</sup>	1.83 <sup>ns</sup>	3475.4 <sup>ns</sup>	2.71 <sup>ns</sup>	2.16*	0.059 <sup>ns</sup>	0.024 <sup>ns</sup>
Y×I	3	0.125 <sup>ns</sup>	4234.7**	0.331 <sup>ns</sup>	7926.3**	0.521 <sup>ns</sup>	0.253 <sup>ns</sup>	0.012 <sup>ns</sup>	0.127*
Y×T×I	6	0.161 <sup>ns</sup>	3286.4**	0.754*	2670**	0.684 <sup>ns</sup>	0.444 <sup>ns</sup>	0.032 <sup>ns</sup>	0.076 <sup>ns</sup>
Pooled Error	36	0.313	272.5	0.229	357.2	0.297	0.218	0.024	0.036

\*  $P \leq 0.05$ ; \*\*  $P \leq 0.01$ ; <sup>ns</sup>: Non-significant; R: Block; df: Degrees of freedom; Y: Year of sowing; T: Sowing date, I: Irrigation treatments.

treatments revealed that higher palmitic acid content was recorded in ceasing irrigation at heading stage in 2008, whereas the maximum safflower oil and protein yield was under normal irrigation at all growth stages which ranked even higher in 2009 compared with 2008 (Figure 4).

### Regression Model

Regression analysis revealed that there was a significant relation between growth degree day (GDD) and oil content and oil

**Table 3.** Fisher's least significant difference test for the effect of irrigation treatments ( $\pm$ SE) on oil, protein, linoleic acid and stearic acid contents of safflower.

Treatments	Oil content (%)	Protein content (%)	Linoleic acid content (%)	Stearic acid content (%)
I <sub>0</sub>	33 $\pm$ 0.51 <sup>a</sup>	19.7 $\pm$ 0.57 <sup>c</sup>	66.63 $\pm$ 0.6 <sup>a</sup>	3.19 $\pm$ 0.12 <sup>c</sup>
I <sub>1</sub>	31.2 $\pm$ 0.55 <sup>d</sup>	21.52 $\pm$ 0.4 <sup>a</sup>	65.22 $\pm$ 0.53 <sup>b</sup>	3.52 $\pm$ 0.11 <sup>a</sup>
I <sub>2</sub>	31.9 $\pm$ 0.54 <sup>c</sup>	20.68 $\pm$ 0.35 <sup>b</sup>	66.06 $\pm$ 0.53 <sup>a</sup>	3.34 $\pm$ 0.14 <sup>b</sup>
I <sub>3</sub>	32.4 $\pm$ 0.51 <sup>b</sup>	20.1 $\pm$ 0.38 <sup>bc</sup>	66.05 $\pm$ 0.6 <sup>a</sup>	3.2 $\pm$ 0.11 <sup>bc</sup>
LSD 5%	0.327	0.708	0.675	0.146

Values followed by the same letter or letters are not significantly different at the 5% level according to LSD (Fisher's least significant difference test). I<sub>0</sub>: Normal irrigation at all growth stages; I<sub>1</sub>: Irrigation ceasing at heading stage; I<sub>2</sub>: Irrigation ceasing at flowering stage, I<sub>3</sub>: Irrigation ceasing at seed filling stage.

and protein yield of safflower. For protein content, this relation was significantly negative (Figure 5). Because of longer growth period for the first sowing date, accumulated GDD value ranked higher compared to the second and third sowing dates of safflower in both years. The results revealed that duration (days) for the maturity of safflower by normal irrigation were highest and it was reduced with limited irrigation stress at heading, flowering, and seed filling stages (Table 1). In this study, the relationship between GDD and safflower fatty acids content was not significant.

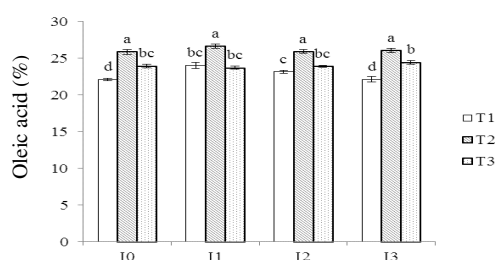
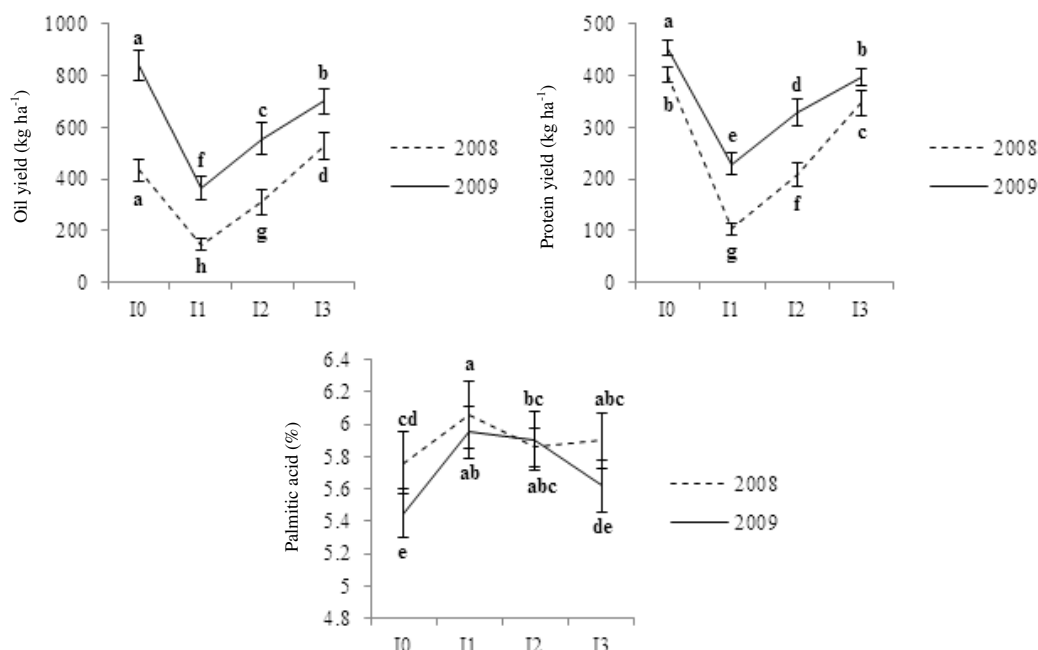


Figure 3. Interaction effects of sowing dates with limited irrigation stress ( $\pm$ SE) on oleic acid content of safflower. Values followed by the same letter or letters are not significantly different at the 5% level LSD (Fisher's least significant difference test). T<sub>1</sub>: April 19<sup>th</sup>; T<sub>2</sub>: May 5<sup>th</sup>; T<sub>3</sub>: May 20<sup>th</sup>; I<sub>0</sub>: Normal irrigation at all growth stages; I<sub>1</sub>: Ceasing irrigation at heading stage; I<sub>2</sub>: Ceasing irrigation at flowering stage, I<sub>3</sub>: Ceasing irrigation at seed filling stage.

## DISCUSSION

### Oil Yield and Content

In 2009, safflower produced significantly higher seed oil content than 2008. The higher oil content in the second year can be explained by better climate factors such as higher precipitations and lower temperatures during stress-sensitive stages of safflower (Figure 1). Late-sowing date reduced oil yield and oil content due to increasing temperature at reproductive phase and, consequently, more rapid development of safflower (Figure 2). It should be noted that the reduction of oil yield is much more affected by the reduction of seed yield than oil content. Emami *et al.* (2011) found that late-sowing date decreased oil content and increased protein content. Hocking and Stapper (2001) stated that high temperature and water stress during seed filling was a major cause of reduced oil content due to late sowing date. Water stress reduced oil content and oil yield (Table 3, Figure 4). Our results were consistent with previous work which demonstrated that water deficit reduced oil content and yield of safflower (Bagheri and Sam-Daliri, 2011; Naderi *et al.*, 2007). Based on the observed results, it can be concluded that a multitude of



**Figure 4.** Interaction effects of year with irrigation treatments ( $\pm$ SE) on oil yield, protein yield and palmitic acid content of safflower. Values followed by the same letter or letters are not significantly different at the 5% level LSD (Fisher's least significant difference test). I<sub>0</sub>: Normal irrigation at all growth stages; I<sub>1</sub>: Ceasing irrigation at heading stage; I<sub>2</sub>: Ceasing irrigation at flowering stage, I<sub>3</sub>: Ceasing irrigation at seed filling stage.

different parameters influence seed oil content and its composition and the observed changes in a measured parameter are not related to a single factor, therefore, a combination of various factors such as enzyme activities, temperature, water deficit, etc, determines the quality of oil seed.

### Protein Yield and Content

Increased protein yield in the second year may have been related to better weather conditions such as higher precipitations and lower temperatures during reproductive stage (Figure 1). Sowing date had a great influence on growth, yield, and quality of seed. The higher protein content produced from T<sub>2</sub> and especially T<sub>3</sub>, sowing dates was due to higher temperatures that occurred during the seed filling period (Figure 2). The results were consistent with finding of Hocking and Stapper (2001); whereas Gao *et al.* (2009) reported late-season high

temperature had little or no effect on protein content. This could be due to differences in genotypes and in the timing and intensity of drought in these studies. Further, Haddadi *et al.* (2010) stated that stressful levels of environmental factors such as temperature and water directly affect oil, protein, and fatty acids contents. Alahdadi *et al.* (2011) reported that drought stress decreased oil and increased protein content of sunflower seed. Increase of seed yield by T<sub>1</sub>, T<sub>2</sub>, and T<sub>3</sub> sowing dates and full irrigation led to high protein yield, however, this increase was statistically more significant in T<sub>1</sub> than the other sowing dates (Figure 4). Ashkani *et al.* (2006) showed that supplementary irrigation can be an important tool to increase seed yield of safflower.

### Fatty Acid Profile

In delayed sowing, high temperature during seed filling stage affected fatty acid

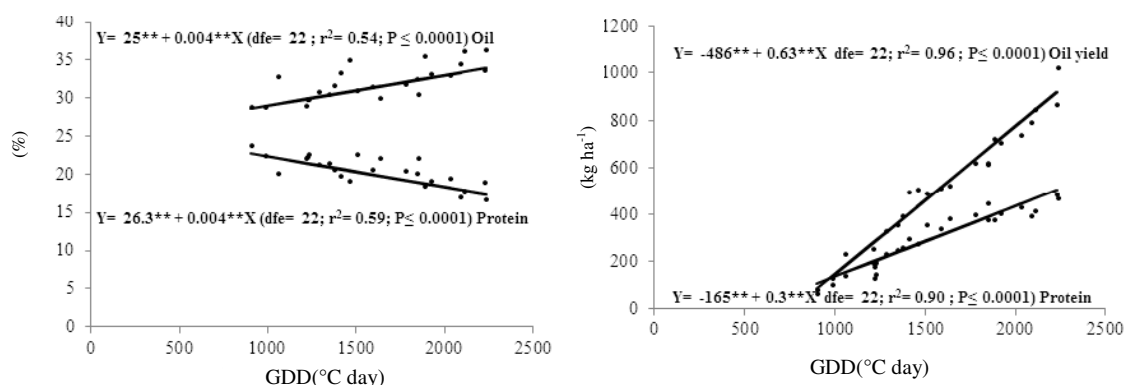


composition of safflower oil (Figure 2). Higher value for oleic and palmitic acid contents were observed on May 5<sup>th</sup> and 20<sup>th</sup> sowing dates. Samanci and O'zkaynak (2003) demonstrated that changes in the composition of the fatty acids of safflower, especially the oleic and linoleic acids, regarding the sowing date, were largely attributed to seasonal weather differences, particularly moisture and temperature, during the growing season. Also, they reported that the highest linoleic acid content and the lowest oleic acid, palmitic acid and stearic acid contents were acquired when the plant was grown in the cooler climate. Gecgel (2007) revealed that the fatty acid composition of safflower in different sowing dates depended on temperature. Bellaloui *et al.* (2009) found that seeds developed under higher temperatures had lower linoleic acid and higher oleic acid. This can be explained by the direct effect of temperature on the activity of desaturase enzymes which convert oleic to linoleic acid. These enzymes are inactivated at a high temperature (Browse and Slack, 1983). Drought stress resulted in decreased linoleic acid content (Table 3). Water stress may cause a reduction in the degree of unsaturation of fatty acids by inhibiting the biosynthesis of polyunsaturated fatty acids and denaturizing activities leading to a reduction in oil content and a change in oil composition

(Ashrafi and Razmjoo, 2010). Ensiye and Khorshid (2010) studied the response of safflower to irrigation regimes and reported that the oleic and linoleic acid contents were reduced by drought stress. Ali *et al.* (2009) stated that the increase or decrease in oleic and linoleic acid contents due to water stress could be different when applied at different growth stages. Limited irrigation stress resulted in increased stearic acid content (Table 3). The results were similar to Hamrouni *et al.* (2001), who reported a significant reduction in the rates of linoleic acid content in parallel with an increase in both stearic and palmitic acids content due to extended drought stress severity. The lower palmitic acid content under drought stress in 2009 in this experiment might have been caused by the higher rainfall and relative humidity during growth stages of safflower compared to 2008 (Figures 1 and 4, Table 3).

### Regression Model

The duration of safflower growth season in 2009 was greater than 2008 (Table 1), due to prolonged vegetative growth period, as affected by lower temperatures. In this research, there was a significant positive relation among growing degree day with oil and protein yield and oil content (Figure 5), indicating that growing degree day plays an



**Figure 5.** Regression relationship between growth degree day with oil content, oil yield, protein content and protein yield of safflower during growing season (pooled values for 2008 and 2009). \*\*: Significant at the 1% probability level.



important role in increasing safflower seed yield. Earlier sowing dates resulted in both compatibility between growth period of crop with low temperature along with enough rainfall in the early season and reducing the negative effect of higher temperature and drought stress in reproductive stages of safflower (Figure 1), as could be seen in some literature (Turner, 2004; Pakrou, 2012).

### CONCLUSIONS

Moisture and temperature are the most important factors affecting crop production. Under arid and semiarid Mediterranean conditions prevailing in Karaj, weather condition is favorable during vegetative growth so that high oil yield, oil content, protein yield and linoleic content could be achieved. Delay in sowing date and drought stress led to more rapid development of the crop and reduction of oil yield, oil content, protein yield and linoleic acid content. The early sowing date and under non-water stress conditions in both years decreased oleic, stearic and palmitic acid contents. Therefore, sowing date and drought influenced safflower seed quality. Safflower breeders could target the development of lines with different protein, oil, oleic acid and linoleic acid contents by managing sowing date, provided that drought stress is avoided at stress-sensitive stages of safflower growth.

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## اثر تاریخ کاشت و آبیاری روی کیفیت دانه گلرنگ

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### چکیده

برای بررسی اثر تاریخ کاشت و آبیاری بر کیفیت دانه ی یک رقم گلرنگ بهاره تحقیقی در سال های ۱۳۸۸ - ۱۳۸۷ در منطقه ی کرج انجام شد. این مطالعه با استفاده از طرح اسپلت پلات بر پایه ی بلوک کامل تصادفی در سه تکرار صورت گرفت که تاریخ های کاشت به عنوان کرت اصلی و سطوح آبیاری به عنوان کرت فرعی بودند. نتایج نشان داد که بیشترین مقدار عملکرد روغن، میزان روغن، عملکرد پروتئین و میزان لینولئیک اسید دانه های گلرنگ در تاریخ کاشت ۱ اردیبهشت ماه و تحت شرایط بدون تنش آبیاری بدست آمد. بالاترین میزان پروتئین، اولئیک اسید و پالمیتیک اسید دانه به ترتیب از تاریخ کاشت ۱۶ و ۳۱ اردیبهشت ماه و با قطع آبیاری در مرحله ی طبق دهی، گلدهی و پرشدن دانه ها کسب شد. تحت شرایط خشک و نیمه خشک مدیترانه ای، مانند کرج، تاریخ کاشت دیر به همزمانی بیشتر مراحل زایشی و دمای بالاتر هوا منتج شد که باعث برجسته شدن اثرات منفی تنش خشکی نسبت به تاریخ کاشت زود گردید.