Turkish Journal of FIELD CROPS

Turk J
Field Crops
2018, 23(2), 117-126
DOI: 10.17557/tjfc.471666

CHANGES IN FATTY ACID AND PROTEIN OF SAFFLOWER AS RESPONSE TO BIOFERTILIZERS AND CROPPING SYSTEM

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Received: 19.06.2018

ABSTRACT

Intercropping and biofertilizer are thought to be strategies for reducing the risks of agricultural production and enhancing yield and quality in developing sustainable agriculture. Hence, two field experiments were carried out with factorial arrangement based on a randomized complete block design with three replications, to evaluate the effects of fertility and cropping systems on quality of safflower in 2015 and 2016. Treatments included nutrient levels (100% chemical fertilizers, 60% and 30% chemical + biological fertilizers and no fertilizer) and safflower and faba bean sole cropping, intercropping systems of them with ratios of 1:1 and 2:1. Results revealed that the integrated use of chemical and biofertilizers in intercropped plants caused to increasing yield components of safflower for both years. The highest unsaturated fatty acids, protein and oil content were achieved in 60% chemical plus biofertilizers. Also, between intercropping systems, ratio of 1:1 and the combinative application of chemical and biofertilizers had the highest land equivalent ratio. Values of actual yield loss showed an advantage of 20 to 98 % from intercropping due to recovery made by intercrops. Therefore, it was concluded that intercropping (safflower / faba bean) with integrated use of the reduced chemical and biological fertilizers, improved growth and quality of safflower.

Keywords: Biofertilizer, fatty acid composition, intercropping, land equivalent ratio, protein content.

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) with an annual production of about 6000 tons in Iran, is one of the most important crops with suitable fatty acid composition among oil crops, because it includes very high content of monounsaturated (oleic acid) or polyunsaturated (linoleic acid) fatty acids, which helps to reduce the cholesterol level in blood, to maintain the health of heart and to prevent the occurrence of cardiovascular diseases (Knowles, 1989; Velasco and Fernandez, 2001; FAO, 2017). The fatty acid composition of safflower oil includes about 6 to 8% palmitic acid (C16:0), 2 to 3% stearic acid (C18:0), 16 to 20% oleic acid (C18:1), and 71 to 75% linoleic acid (C18:2). Also, the seeds contain nearly 12–24% protein content (Velasco and Fernandez, 2001). Many factors influence oil yield and fatty acid composition such as cultivar type, climate, morphology, physiology and plant management during plant growth (density, irrigation, planting time and fertilization) (Sabzalian et al., 2008). Increasing concern about climate change and environmental impacts require transformation of actual cropping systems by focusing on enhanced sustainability. Intercropping, which is defined as growing more than one species simultaneously in the same field during a growing season, is considered one important strategy in developing sustainable production systems, particularly systems that aim to limit external inputs (Jahansooz et al., 2007; Zhang et al., 2008). The range of benefits identified from intercropping two or more species include higher productivity and profitability per unit area, improved soil fertility, increased efficiency of resources, reduced damage caused by pests, diseases and weeds (Oelbermann and Echarte, 2011). Most of the advantages of intercropping are obtained by application of legume species (Manjith Kumar et al., 2009). The quantity and quality of crop production in the intercropping of oil crops with legumes are increased due to the effective use of the available resources (Singh Rajesh et al., 2010). Intercropping of chickpea and safflower resulted in increased crop yield, maximized resource consumption and enhanced productivity of cultivation system (Singh Rajesh et al., 2010; Zafaranieh, 2015). Intercropping systems can change the synthesis of oil and its fatty acids through changing environmental conditions (Tsubo et al., 2005). Abdelkader and Hamad (2015) indicated that safflower / fenugreek intercropping increase oil and protein content of safflower. The excessive use of chemical inputs, has led to a significant increase in global production, but on the other hand, it also causes pollution of water and soil, as well as the loss of non-renewable resources (Jalilian et al., 2012). Therefore, alternative methods should be used. Biological fertilizers contain a dense population of one or more useful soil organisms that improve plant growth and increase the quantity and quality of yield due to increasing of availability to the nutrient requirements of the plant and stimulating the vital activities of soil (Abou-Khadrah et al., 2000). Plant growth promoting rhizobacteria, PGPR (e.g. *Azotobacter chroococcum* as free-living nitrogen fixing bacteria and *Bacillus megatherium* and *Pseudomonas sp*. as phosphate dissolving bacteria) that positively affect growth, yield and yield components of many crops (Timmusk et al., 1999). In some studies, it was clearly revealed that biofertilizer application resulted in high productivity for safflower (Mirzakhani et al., 2009; Seyed Sharifi, 2012). Soleymanifard and Sidat (2011) reported a significant increasing oil and protein content of safflower using 60 kg/ha N with Azospirillum. Also, Seyed Sharifi et al. (2017) reported that the biofertilizer inoculation enhances oil and fatty acids content of safflower seed. The role of grain legumes such as faba bean (*Vicia faba* L.) in an intercropping system is important due to its ability to biological nitrogen fixation as a nutritive source for the entire system. Besides nitrogen fixation, other advantages of faba bean include worldwide use for food and feed, enhancement of soil structure, maintenance of soil productivity and diversification of the agroecosystem in space via intercrops (Kopke and Nemecek, 2010). Therefore, the goals of this research were the evaluation of crop yield, fatty acid composition, oil and protein content of safflower as affected by cropping systems, chemical and biological fertilizers application in the northwestern Iran.

MATERIALS AND METHODS

Site description, experimental design and treatments

Two field experiments were conducted during 2015 and 2016 growing seasons at Research Farm of Tabriz University (Latitude, 46° 17′ E; 38° 05′ N, Altitude 1360 m, East Azarbaijan Province, Tabriz -Iran). Weather conditions of the experimental site including the monthly precipitation and mean air temperature are presented in Figure 1. The Factorial set of treatments was arranged within Randomized Complete Block Design (RCBD) with three replications. The first factor was cropping systems including: safflower sole cropping (C1), faba bean sole cropping (C2), intercropping of safflower / faba bean with the ratios of 1:1 (C3) and 2:1 (C4). The second factor consisted of different nutrient levels of application of 100% recommended chemical fertilizer (F1), 30% chemical + biological fertilizers (F2), 60% chemical + biological fertilizers (F3) and control or no fertilizer (F0). Fertilizers consisted of triple superphosphate (50 kg/ha), urea (75 kg/ha), biofertilizers of Phosphate Barvar-2 (contain phosphate dissolving bacteria) and Azoto Barvar-1 (contain free living nitrogen fixing bacteria). The soil texture of experiment site was sandy-loam (20% silt, 15% clay, 65% sand) with 0.15% total nitrogen, 1.1 ds/m electrical conductivity, 0.76% organic carbon, 16 mg/kg phosphorus, 290 mg/kg potassium and pH=7.4.

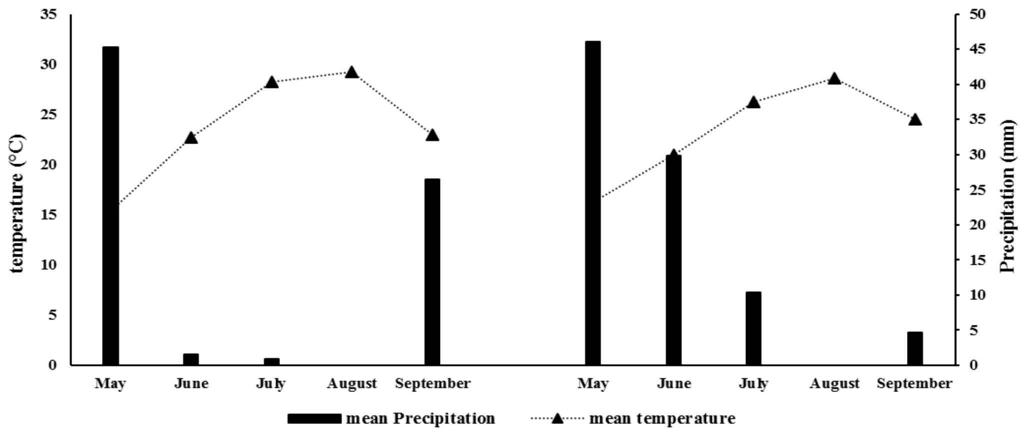

Figure 1. Means of temperature and precipitation during the two growing seasons 2015 (left) and 2016 (right) at the experimental station, Faculty of Agriculture, Tabriz University, Iran.

Sowing dates of safflower and faba bean were 16 May, 2015 and 14 May, 2016. Triple superphosphate was applied at sowing time, but urea was used as equal split at the stages of stem elongation and heading. After preparing of solution for Phosphate Barvar-2 and Azoto Barvar-1, seeds for inoculation, were sprayed with this solution, immediately, before sowing. Plant densities of safflower (cv. Goldasht) and faba bean (local variety) were 40 and 20 plant/m^2, respectively. Plot size in each cropping system was different and consisted of different number of rows with 4 m length, and 50 cm inter row spacing. For 1:1 cropping system, two rows of safflower were intercropped with one row of faba bean (with plot sizes of 4×3 m^2). Also, four rows of safflower were intercropped with two rows of faba bean for 2:1 cropping system (with plot sizes of 4×6 m^2). Four rows of each crop were sown in sole cropping with plot sizes of 4×2 m^2 (Figure 2). The first irrigation was done after seed sowing. Subsequent irrigations was carried out according to the weather conditions and plant requirements (once irrigation per seven days). Weed control was carried out as required. Safflower was harvested when most of the leaves turn a brown color and very little green remains on the bracts of the latest flowering heads. For all treatments, safflower and faba bean were harvested in the first week of September and the first week of August in both years, respectively.

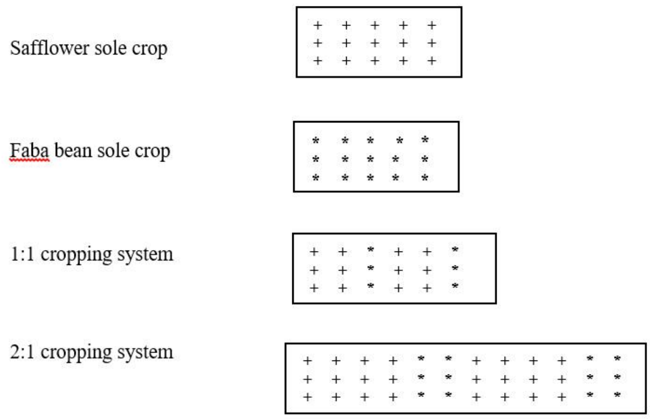

Figure 2. Schematic diagram of different cropping systems between safflower (+) and faba bean (*)

Measurement of safflower parameters

Safflower was harvested at physiological maturity by cutting 10 plants randomly from each plot to determine head number per plant, seed number per head and 1000 seed weight. Also, plants in 2 m^2 of each plot were harvested and then, seed and biological yields per unit area were recorded. The seed nitrogen content was estimated by Kjeldahl method. The protein amount was calculated by multiplying total nitrogen content with factor 6.25 (AOAC, 1960). Dried seeds of safflower were grinded and seed oil was extracted according to the AOCS (1993) method with hexane in soxhlet extractor. Oil yield and oil harvest index were calculated by following Formulas:

$$\text{Oil yield} = \%\ \text{oil} \times \text{seed yield} \quad (1)$$

$$\text{Oil harvest index} = (\text{oil yield} / \text{biological yield}) \times 100 \quad (2)$$

The oil samples were transformed to its fatty acid methyl esters (FAME) according to the AOCS (1993) method. Determination of fatty acids was performed by gas chromatography method. The apparatus for gas chromatography Varian CP-3800 was equipped with a FID detector (Flame Ionization Detector) and a column (CPsill-88, 100m×0. 25mm ×0/2μm). Helium gas was used as carrier gas with a pressure of 5 bar and a flow rate of 1.3 ml / min. The temperature of the injection site was set at 250 °C. After injection of each sample into the gas chromatography system, the curves were plotted and the retention time related to each fatty acid was compared with the curve of standard fatty acids and its retention time. The type and value of fatty acids were determined.

Evaluation of intercropping

The intercropping success was evaluated by computation of land equivalent ratio (LER) that has usually been recognized as an index of intercropping advantage. The LER is described as follows:

$$LER = (Yab / Yaa) + (Yba / Ybb) \quad (3)$$

Where Yab and Yba are the yields of “a” and “b” species as intercrops, respectively, and Yaa and Ybb are the yields of “a” and “b” species as sole crops, respectively. While a LER is greater than one, intercropping has the advantage and if it is less than one, the sole cropping is preferred (Vandermeer, 1989).

Another index used in intercropping is actual yield loss (AYL) that can be calculated according to following formula (Banik, 1996).

$$AYL = AYLa + AYLb \quad (4)$$

$$AYLa = [(Yab/Zab) / (Yaa/Zaa)]-1 \quad (5)$$

$$AYLb = [(Yba/Zba) / (Ybb/Zbb)]-1 \quad (6)$$

Where Zab and Zaa represent the sown proportion of “a” in intercropping and sole cropping, respectively. Zba and Zbb represent the sown proportion of “b” in intercropping and sole cropping, respectively.

Statistical analysis

Combined analysis of variance was performed using MSTAT-C software. Means of treatments were compared with the Duncan's multiple range test at the 5% probability level. The data showed normal distribution and no transformation was required.

RESULTS AND DISCUSSION

Yield components of safflower

Head number per plant and seed number per head were significantly influenced by fertilizer and cropping systems. Also, year and interaction of year × fertilizer had significant effect on these traits (P≤0.01) (Table 1). Maximum and minimum values of both traits were recorded in 60% chemical plus biological fertilizers and no application of fertilizers, respectively. Further, number of head per plant and seed per head were increased in safflower / faba bean intercropping (1:1), compared to sole cropping (Table 2). On the basis of Table 1, the effects of year, cropping systems, fertilizers and the interaction of cropping systems × fertilizers were significant on 1000-seed weight. The highest value (46.05 g) was obtained in cropping system of 1:1 with application of 60% chemical plus biological fertilizers, while the lowest (37.22 g) was observed at sole cropping of safflower with no application of fertilizers (Table 3). Head number per plant, seed number per head and 1000-seed weight, as safflower yield components, were influenced by cropping systems and fertilizer treatments. In general, integrated application of biofertilizers with chemical fertilizers at the recommended amount (60%) can cause to increasing seed yield and its components via supplement of sufficient amount as same as positive effect on macro and micro element absorption such as N, P, K and Zn, Fe, respectively and also, affected improving water uptake due to developing roots (Narula et al., 2000). Presumably, utilizing 60% chemical fertilizer, provided better nourishment situation to activity and reproducing *Azotobacter* and *Bacillus* sp, because these bacteria require to chemical elements to growing and developing. Farnia and Moayedi (2014) showed that there was a significant difference between seed inoculation by N and P biofertilizers, and non-inoculation treatment, concerning the weight of one thousand seeds. Some recent findings also indicated that the potential for safflower yield components increasing through intercropping of legumes with safflower (Zafaranieh, 2015; Jalilian et al., 2017). These results confirm our findings. Progress in yield components may be due to increasing light interception by safflower canopy as intercropped with faba bean plants. Then, improvement the light absorption will enhance photosynthesis and produce more photosynthetic materials, which ultimately increase the seed yield components.

Table 1. Two-year analysis of variance (mean square) for yield, yield components of safflower and faba bean affected by different fertilizer treatments and cropping systems

S.O.V	df	Safflower: Head per plant	Seed per head	1000 seed weight	Seed yield	Biological yield	Protein content	Faba bean: Seed yield	Biological yield
Y	1	32.940**	502.128**	355.511**	1810961.2**	1750835.794**	18.41**	2217519.39**	1768792.27**
Y*B	4	0.282**	19.841**	5.549**	5119.36**	2154.414	0.85**	22617.81**	101690.85**
C.S	2	7.658**	251.137**	86.040**	1760673.41**	1290012.537**	0.69	9040738.59**	645303.33**
F	3	15.775**	307.189**	79.132**	554660.03**	1434198.256**	56.89**	768581.1**	962169.74**
C.S. * F	6	0.117	1.657	2.633**	18793.1 **	13436.781**	0.034	1094.21	5284.17
Y * C.S	2	0.133	1.966	0.574	2228.04	10921.763	0.20	7609.77	1521.20
Y* F	3	1.444**	10.449**	0.095	4112.83*	15903.881**	0.289	49088.82**	1134.35
Y*F*C.S	6	0.088	0.829	0.289	1153.13	2462.976	0.058	2468.05	1445.83
E	44	0.051	0.727	0.349	1025.68	3511.821	0.113	2378.04	2405.13
C.V. (%)		3.15	1.84	1.44	3.34	7.50	4.98	3.00	9.78

Notes: *Statistically significant at p ≤ 0.05. **significant at p ≤ 0.01. Y: year, B: block, C.S.: cropping system, F: fertilizer and E: error.

Seed and Biological yields

The results indicated that seed and biological yields of safflower and faba bean were significantly influenced by year, cropping systems and fertilizers (P≤0.01) (Table 1). In the second year of the experiment, these traits were higher compared to the first year (Table 2). During the growing season, the mean temperature in the second year was lower than in the first year, and in the second year, the mean rainfall was higher than in the first year, this could be a reason for the differences between years. It seems to weather conditions of the second year were more favorable for growth and production of safflower and faba bean. Seed and biological yields of safflower and faba bean were improved as fertilized with the integrated application of chemical and biological fertilizers (Table 2). The highest seed yield (2792.92 kg ha^{-1}) and biological yield (8319.15 kg ha^{-1}) of safflower were obtained from sole cropping of safflower along with application of 60% chemical plus biological fertilizers. In versus, the lowest values for both traits were achieved in intercropping (2:1) with no application of fertilizers (Table 3). Also, in both years, seed and biological yield of faba bean was significantly higher under sole cropping than under intercropping systems (Table 2). This was mainly attributed to interspecific competition between safflower and faba bean for light, water and nutrients. Observed results are consistent with reports by Fuente et al. (2014), who observed lower soybean yield in intercrop with sunflower in temperate regions. Layek et al. (2015) also reported that yield of soybean intercropped with maize in the sole cropping was higher than the intercropping system.

Table 2. Mean comparison of yield, yield components, oil and protein of safflower and yield of faba bean affected by cropping systems and fertilization in the 2015 and 2016 cropping seasons

Treatment	Safflowe							Faba bean	
	Head per plant	Seed per head	1000 seed weight(g)	Seed yield ($kg.ha^{-1}$)	Biological yield ($kg.ha^{-1}$)	Oil yield ($kg.ha^{-1}$)	Oil Harvest index (%)	Seed yield ($kg.ha^{-1}$)	Biological yield ($kg.ha^{-1}$)
Year									
2015	6.51 b	43.79 b	38.86 b	1978.78 b	7019.01 b	595.79 b	8.37 b	1447.91 b	5586.59 b
2016	7.87 a	49.07 a	43.31 a	2258.26 a	7330.87 a	737.43 a	9.93 a	1798.90 a	5955.52 a
cropping system									
C1	6.57 b	43.38 c	39.27 c	2642.22 a	7959.42 a	829.70 a	10.37 a	2320.73 a	6484.1 a
C3	7.67 a	49.82 a	43.05 a	2102.82 b	7167.48 b	664.16 b	9.20 b	1165.22 c	5194.67 c
C4	7.33 a	46.10 b	40.92 b	1610.51 c	6397.91 c	505.98 c	7.87 c	1384.26 b	5510.44 b
Fertilizer									
F0	6.18 d	41.52 d	38.52 d	1932.47 d	6822.51 d	538.55 d	7.78 c	1367.72 d	5375.12 d
F1	7.66 b	48.89 b	42.10 b	2195.22 b	7336.29 b	742.09 b	10.01 a	1721.17 b	5874.86 b
F2	6.67 c	44.66 c	40.37 c	2056.59 c	7082.67 c	609.68 c	8.50 b	1559.73 c	5688.88 c
F3	8.25 a	50.66 a	43.34 a	2289.89 a	7458.27 a	776.11 a	10.30 a	1844.98 a	5980.02 a

Notes: Different letter (s) indicates significant difference at $p \leq 0.05$.

Table 3. Interactive effect of cropping systems and fertilization on 1000 seed weight, seed and biological yield, oil yield and harvest index of safflower in two years

Treatment		1000 seed weight (g)	Seed yield (kg.ha^{-1})	Biological yield (kg.ha^{-1})	Oil yield (kg.ha^{-1})	Oil Harvest index (%)
C1	F0	37.22 i	2505.73 d	7557.95 d	696.64 e	9.20 cd
	F1	40.21 fg	2689.70 b	8111.10 b	908.97 b	11.19 a
	F2	38.72 h	2580.53 c	7849.48 c	768.40 d	9.77 c
	F3	40.95 ef	2792.92 a	8319.15 a	944.78 a	11.34 a
C3	F0	39.53 gh	1816.08 g	6835.19 g	506.99 gh	7.39 f
	F1	44.48 b	2230.10 e	7346.44 e	753.15 d	10.24 b
	F2	42.15 cd	2022.35 f	7077.02 f	599.08 f	8.44 e
	F3	46.05 a	2342.75 e	7411.28 de	797.41 c	10.74 b
C4	F0	38.81 h	1475.61 i	6074.39 i	412.02 i	6.77 g
	F1	41.62 de	1665.85 h	6551.33 h	564.15 g	8.60 de
	F2	40.25 fg	1566.90 hi	6321.51 hi	461.57 h	7.29 fg
	F3	43.01 c	1733.70 gh	6644.40 g	586.16 fg	8.81 d

Notes: Different letter (s) indicates significant difference at $p \leq 0.05$.

In due attention to the results of Table 2, biofertilizers application caused to increasing seed and biological yield in compared with control. Our research results showed that using biofertilizer could not only enhanced seed yield but also exceptionally diminished utilizing chemical fertilizer. These results agree with those reported by Soleymanifard and Siadat (2011), Mirzakhani et al. (2009) and Mirzaei and Vazan (2013) in safflower. Biofertilizers play an important role in reduction of the negative environmental effects and increasing the yields of agricultural products. Studies of Raei et al. (2017) on soybean/maize intercropping showed that biofertilizers application and N chemical fertilizer improved yield and yield components.

Oil and Protein contents

According to Tables 1 and 4, oil and protein contents were significantly affected by year and fertilizer ($P \leq 0.01$). These traits were uninfluenced by cropping systems. Maximum oil content (33.81%) was observed with applying of 60% chemical plus biological fertilizers followed by 100% chemical fertilizer (33.73%). Also, application of 60% chemical plus biofertilizers increased protein content of safflower (Table 5). It was clearly shown that oil and protein contents were higher in the second year than that of the first year (Table 5), due to more favorable of climatic factors (less temperature and more rainfall) of second growth season for faba bean and safflower production. In the second year, suitable temperature during seed filling period, probably, lead to produce higher oil and protein contents. It found that high temperature during seed development produce seeds with low oil concentration (Pritchard et al., 2000). Integrated application of 60% chemical plus biological fertilizers caused to producing seeds with high oil (33.81 %) and protein (18.39 %) contents. Shehata and El-khawas, (2003) and Akbari et al. (2011) reported similar findings in confirmation that biofertilizer application resulted in improved of oil and protein contents of seed. In general, it can be concluded that synergistic effect of biofertilizers, specially, Azoto Barvar-1 and Phosphate Barvar-2, may have been resulted from its ability to increase the availability of N and P of soil, which causes to improve seed quality.

Oil yield and Harvest index of oil

The results in Table 4 showed that the effects of years, cropping systems, fertilizers and the interactions of cropping systems × fertilizers were significant on oil yield and oil harvest index ($P \leq 0.01$). Combinative application of chemical and biological fertilizers in sole cropped and intercropped plants caused to improving oil yield of safflower (Table 3). Oil harvest index was enhanced with applying of biological fertilizers plus 60% chemical fertilizers (11.34%) followed by 100% (11.19%) chemical fertilizer (Table 2). The application of 60% chemical plus biological fertilizers significantly enhanced oil yield due to chiefly augmentation in seed yield. Mean oil yield and oil harvest index were higher in the second year compared to the first year (Table 2). This could be attributed to higher rainfall and lower temperature in 2016 relative to 2015. Krol and Paszko (2017) found that there is a relationship between climatic parameters and oil content, oil yield and fatty acid composition. In confirmation of our results, utilization of different strains of *Azotobacter* in soybean increased oil yield (Kumar, 1994). Similar results have been reported on safflower by Mohsennia and Jalilian (2012); Mirzaei and Vazan (2013).

Table 4. Two-year analysis of variance (mean square) for oil and fatty acid composition of safflower affected by different fertilizer treatments and cropping systems.

S.O.V	df	Palmitic acid (C16:0)	Stearic acid (C18:0)	Oleic acid (C18:1)	Linoleic acid (C18:2)	linolenic acid (C18:3)	Oil content	Oil yield	Oil Harvest index
Y	1	0.731**	0.008	13.451**	230.624**	0.0185**	117.68**	1033176.80**	111.58**
Y*B	4	0.088	0.006	1.231	1.137	0.0009	7.11**	14447.71**	2.442**
C.S	2	0.003	0.033	0.119	0.800	0.0012	0.029	3137656.17**	424.95**
F	3	9.476**	0.004	87.206**	143.401**	0.0214**	165.75**	646101.24 **	66.43**
C.S. * F	6	0.001	0.040	0.037	0.858	0.0002	0.083	27385.39 **	2.44**
Y * C. S	2	0.001	0.010	0.305	0.566	0.0003	0.130	1764.96	0.321
Y* F	3	0.010	0.043	0.574	1.037	0.001	0.320	1510.09	0.142
Y*F*C.S	6	0.002	0.013	0.055	0.498	0.0001	0.117	339.65	0.046
E	44	0.092	0.090	0.780	2.325	0.0004	0.207	556.92	0.105
C.V. (%)		5.05	12.30	7.95	2.10	7.01	1.46	8.12	2.28

Notes: *Statistically significant at $p \leq 0.05$. **significant at $p \leq 0.01$. Y: year, B: block, C.S.: cropping system, F: fertilizer and E: error.

Table 5. Mean comparison of fatty acid composition of safflower affected by fertilization in the 2015 and 2016.

Treatment	Palmitic acid (C16:0)%	Stearic acid (C18:0)%	Oleic acid (C18:1)%	Linoleic acid (C18:2)%	linolenic acid (C18:3)%	Protein content (%)	Oil content (%)
Year							
2015	8.36 a	2.446 a	11.68 b	75.45 b	0.27 b	16.45 b	29.94 b
2016	6.48 b	2.434 a	12.84 a	76.33 a	0.31 a	17.46 a	32.49 a
Fertilizer							
F0	8.98 a	2.459 a	11.95 c	74.81 c	0.25 c	14.77 d	27.79 c
F1	6.92 c	2.443 a	12.65 b	76.69 a	0.31 a	17.42 b	33.73 a
F2	8.31 b	2.453 a	11.82 c	75.69 b	0.26 b	16.25 c	29.54 b
F3	6.01 d	2.428 a	13.13 a	76.97 a	0.32 a	18.39 a	33.81 a

Notes: Different letter (s) indicates significant difference at $p \leq 0.05$.

Fatty acid composition

In attention to results of Table 4 showed that palmitic, oleic, linoleic and linolenic acids concentrations were significantly influenced by year and fertilization treatments. Also, treatments had no significant effect on stearic fatty acid. The highest values of linoleic acid (76.97-76.69%) and linolenic acid (0.32-0.31%) were observed in 60% chemical plus biological fertilizers and 100% chemical fertilizer application. In contrast, the lowest levels of linoleic acid (74.81%) and linolenic acid (0.25%) were shown in control. Furthermore, the highest value for oleic acid content (13.13%) was obtained from the use of 60% chemical plus biological fertilizers (Table 5). On the other hand, maximum and minimum values of saturated fatty acid of palmitic were observed in control and 60% chemical plus biological fertilizers application, respectively (Table 5). Additionally, it was revealed that palmitic content for combinative fertilizing was decreased as chemical fertilizers application increased. The unsaturated fatty acids were affected by integrated application of biological and chemical fertilizers. Therefore, biofertilizers application instead of a part of chemical recommended fertilizers not only significantly improved linoleic acid (as a very desirable fatty acid for human diet), but also reduced unsuitable side effects on environment. The fatty acid values obtained in this research are alike to those described by Arslan (2007) and Vosoughkia et al. (2011) in safflower. Knowles (1989) reported that cultivars with high levels of linoleic acid have a high nutritional value. Due to the obligation of linoleic and linolenic fatty acids for the human body (essential fatty acids) and its high level in safflower oil, it can be stated that this oil has a high nutritional value similar to olive oil (Purdy, 1985). Also, the saturated fatty acids were reduced by integrated application of biological and chemical fertilizers. Inoculation of soybean and safflower seeds with biofertilizers improves quality of oil by increasing unsaturated fatty acids and reducing saturated fatty acids (Seyed Sharifi et al, 2017). In addition, Gao et al. (2010) and Luis et al. (2013) obtained similar results in their studies with application of fertilizers to the increase of unsaturated fatty acids for canola and soybean, respectively. Seyed Sharifi (2016) noted that the use of biological fertilizers could be useful in enhancing quality of seed oil by observing a significant increase in unsaturated fatty acids of soybean oil. Based on mean comparisons, the saturated fatty acids were reduced in the second year, in comparison with the first year, while unsaturated fatty acids were increased. These results were in accordance with that reported by Cosge et al. (2007). Temperature changes can affect the composition of fatty acids during the growing season (Lajara et al., 1990). Krol and Paszko (2017) found that there is a relationship between climatic parameters and oil content and fatty acid composition.

Land equivalent ratio (LER)

Data recorded in Table 6 revealed that, land equivalent ratio was above 1.00 in all intercropping systems for both years. Moreover, LER values of the second year were higher than that of the first year in all of treatment combinations. The highest LER (1.41) was obtained from cropping system of 1:1 with applying of 60% chemical plus biological fertilizers in 2016 and the lowest (1.10) was achieved in safflower with faba bean intercrop (2:1) for control in 2015 (Table 6). As can be seen from Table 6, it is clear that partial LER of safflower (LER_s) in 1:1 cropping systems with 60% chemical plus biological fertilizers was higher than those of other treatment combinations in both years. This verifies the advantage of intercropping systems to gain more production from the same area of land compared to the same unit of area in which sole crop is applied. Any intercropping system evaluates the utility of different intercrop combinations. The high level of land use efficiency indicates the usefulness of intercropping compared to sole cropping due to better use of available resources by two plants and morphological and physiological differences between them in cropping system. According to the results of this research, safflower and faba bean are cooperative and beneficial crops to be intercropped together. Similar results were recorded by Jalilian et al. (2017) when intercropped safflower with bitter vetch and Raei et al. (2015) when intercropped potato with green bean. Inoculation of intercropped plant species with biofertilizers could increase yield as a result of more efficient use of resources by crops. In this research, intercrops that inoculated with biofertilizers improved the seed yield of safflower and faba bean, which supported by the findings of Jalilian et al. (2017).

Table 6. Effect of cropping systems and fertilization on land equivalent ratio (LER), partial LER of safflower (LERs), actual yield loss (AYL) and partial AYL of safflower (AYLs) in two years.

Treatment		AYLs		AYL		LER		LERs	
		1:1	2:1	1:1	2:1	1:1	2:1	1:1	2:1
2015	**F0**	0.41	-0.14	0.20	0.44	1.11	1.10	0.70	0.57
	F1	0.64	-0.09	0.59	0.61	1.29	1.17	0.82	0.61
	F2	0.52	-0.11	0.40	0.53	1.20	1.14	0.76	0.60
	F3	0.67	-0.08	0.69	0.66	1.35	1.19	0.84	0.62
2016	**F0**	0.49	-0.10	0.50	0.70	1.24	1.20	0.74	0.60
	F1	0.67	-0.06	0.78	0.90	1.39	1.28	0.83	0.63
	F2	0.61	-0.08	0.66	0.78	1.33	1.23	0.80	0.61
	F3	0.68	-0.07	0.83	0.98	1.41	1.30	0.84	0.62

Actual yield loss (AYL)

AYLs had positive values in 1:1 cropping systems while the negative values were obtained from cropping system of 2:1 (Table 6). Actual yield loss index gave more accurate information than the other indices on inter and intraspecific competitions and the behavior of each species in intercropping systems (Banik et al., 2000). Partial actual yield loss also demonstrates the proportionate yield loss or gain by its sign and as its value (Dhima et al., 2007). Thus, there was a 68% (AYLs = + 0.68) increase in yield of safflower in 1:1 cropping systems with 60% chemical plus biological fertilizers, when compared to its sole crop yield. However, in 2:1 cropping systems, the AYLs ranged from -0.14 to -0.06 indicating a yield loss of 14-6%, compared to sole crop yield. In both years, total actual yield loss values were more than zero in all intercrops which suggests an advantage of intercropping over sole crops (Table 6).

CONCLUSION

In general, the results indicate that cropping system and fertilizer treatments significantly affected yield components of safflower. According to the findings, seed and biological yield of faba bean and safflower, oil and protein contents of safflower were improved as fertilized with the integrated application of chemical and biological fertilizers. Values of unsaturated fatty acids were increased as biofertilizers integrated with chemical fertilizers, while saturated fatty acids were decreased. In both years, the LER was above 1.00 and AYL was more than zero in all intercropping systems. Finally, it was concluded that integrated application of chemical with biological fertilizers, enhanced growth and quality of safflower, with a reduction of chemical fertilizers consumption to prevention the destruction of environment and help to development of environmental-friendly cropping systems.

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