Grain filling and fatty acid composition of safflower fertilized with integrated nitrogen fertilizer and biofertilizers

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Abstract – The objective of this work was to evaluate the effects of the integrated application of nitrogen fertilizer and biofertilizers on the yield, grain filling period, and composition of fatty acids of safflower (*Carthamus tinctorius*). Split-plot experiments were carried out during the 2011 and 2012 crop seasons. The treatments consisted of seed inoculation with plant growth-promoting rhizobacteria (*Azotobacter chroococcum* strain 5, *Azospirillum lipoferum* strain F, and *Pseudomonas putida* strain 186) in the subplots, including a control without seed inoculation; and of the application of N fertilizer at different rates (60, 120, and 180 kg ha^{-1} urea) in the main plots, including a control without N. The highest grain yield, grain filling period, and effective grain filling period were obtained by the application of 180 kg ha^{-1} urea and by seed inoculation with *P. putida*. The application of high N rates and *P. putida* inoculation resulted in 25.66% increase of the potential rate of grain filling. Biofertilizer inoculation in seed reduced the contents of saturated fatty acids (palmetic and stearic acids) and increased the contents of unsaturated fatty acids (linoleic, linolenic, and oleic acids). The suitable amount of N fertilizer (between 120 and 180 kg ha^{-1} urea) can improve plant growth, and the quantity and quality of oil in seeds treated with *P. putida* in safflower plants.

Index terms: *Carthamus tinctorius*, *Pseudomonas putida*, oil quality, PGPR, fatty acids, seed-inoculation.

Enchimento de grãos e composição de ácidos graxos em plantas de cártamo adubadas com fertilizante nitrogenado e biofertilizantes

Resumo – O objetivo deste trabalho foi avaliar os efeitos da aplicação integrada de fertilizante nitrogenado e biofertilizantes sobre o rendimento, o período de enchimento de grãos e a composição de ácidos graxos de cártamo (*Carthamus tinctorius*). Os experimentos foram conduzidos em parcelas subdividas, durante as safras 2011 e 2012. Os tratamentos consistiram da inoculação de rizobactérias promotoras do crescimento de plantas (*Azotobacter chroococcum* estirpe 5, *Azospirillum lipoferum* estirpe F e *Pseudomonas putida* estirpe 186) nas sementes, nas subparcelas, com um controle sem inoculação; e da aplicação de diferentes níveis de adubação nitrogenada (60, 120 e 180 kg ha^{-1} de ureia) nas parcelas principais, com um controle sem aplicação de N. O maior rendimento de grãos, o maior período de enchimento de grãos e o período efetivo de enchimento de grãos foram obtidos com a aplicação de 180 kg ha^{-1} de ureia e com a inoculação de *P. putida* nas sementes. A aplicação de altas doses de N e a inoculação com *P. putida* resultaram no aumento de 25,66% da taxa potencial de enchimento de grãos. A inoculação de biofertilizantes nas sementes reduziu o teor de ácidos graxos saturados (ácidos palmítico e esteárico) e incrementou os ácidos graxos insaturados (ácidos linoleico, linolênico e oleico). A aplicação da quantidade adequada de adubação nitrogenada (120 a 180 kg ha^{-1} de ureia) pode melhorar o crescimento de plantas e a quantidade e a qualidade do óleo das sementes tratadas com *P. putida*, em plantas de cártamo.

Termos para indexação: *Carthamus tinctorius, Pseudomonas putida,* qualidade do óleo*,* PGPR*,* ácidos graxos, inoculação em sementes.

Introduction

Safflower (*Carthamus tinctorius* L.) is one of the most important oil seed all over the world. It has a high need of nitrogen (N) due to this nutrient multidimensional effects on the growth and development of this crop than other nutritional elements (Kulekci et al., 2009). Golzarfar et al. (2012) suggested that nutrient management is one of the critical inputs to achieve a high productivity of safflower. Hence, there is a need to improve this major component of the production technology to get a higher safflower production.

Safflower N requirements depends on the amount of N in the soil, soil productivity, and preceding crop (Siddiqui & Oad 2006). Soleymanifard & Sidat (2011) suggested that yield and yield attribute of safflower increased by the increment of N application rate. This element is also the major macronutrient that determines the rate and period of grain filling. Final grain weight was related to grain filling rate, grain filling duration, and their interactions (Sadras & Egli, 2008). Borrás et al. (2004) found that lack of assimilate supply, during the grain filling period, could result in a dramatic decline of grain weight. Dordas & Sioulas (2008) reported that higher rates of N application increase the photosynthetic processes, leaf area production, and leaf area duration, as well as the grain filling period.

Although N is the key element for the increasing of safflower productivity, and, consequently for this crop production increment per unit of area, large rates of N fertilizer loss to the environment could cause a serious environmental problem, such as groundwater contamination. In such a situation, the reduction of N application rates to an optimized level, with the application of biofertilizers, can reduce the need for chemical fertilizers, decrease adverse environmental effects, increase soil organic matter, improve soil properties, and enhance crop yield. Therefore, in the development and implementation of sustainable agriculture techniques, biofertilization is important to alleviate environmental pollution and deterioration of nature (Namvar & Khandan, 2015).

Safflower oil quality is determined by oil composition of saturated and unsaturated fatty acids. Nasim et al. (2012) concluded that N is the most important element to increase grain oil content. They stated that increasing N rates reduced seed oil percentages, but increased seed yield, and, consequently, increased oil yield per unit area. Silva et al. (2013) reported that biofertilizer inoculation in soybean enhanced oil content and unsaturated fatty acids, while decreased saturated fatty acids. Moreover, Mirzakhani et al. (2009) showed that safflower yield increased in plants inoculated with *Azotobacter*.

The determination of safflower response to N application and seed inoculation by biofertilizers is very important to maximize yield and economic profitability of safflower production. In addition, it seems that there is little investigation about the combined effects of N fertilization and biofertilizer on yield, grain filling period, and composition of fatty acids of safflower.

The objective of this work was to evaluate the effects of the integrated application of nitrogen fertilizer and biofertilizers on the yield, grain filling period, and composition of fatty acids of safflower (*Carthamus tinctorius*).

Materials and Methods

Field experiments were conducted in a randomized complete block design, in split plots with three replicates, during 2011 and 2012 crop seasons. The treatments were N applications at four rates (no N application as control, and 60, 120, and 180 kg ha^{-1} urea), assigned to the main plots; and seed treated by biofertilizer inoculations, assigned to the subplots, which were: no inoculation as control, and inoculation with *Azotobacter chroococcum* strain 5, *Azospirillum lipoferum* strain F, and *Pseudomonas putida* strain 186. The area is located at 38°15'N and 48°15'E, 1,350 m altitute. Mean temperature and precipitation during safflower growing seasons of 2011 and 2012 is presented in Figure 1. The studied area soil is an Entisol with a silty loam texture. Other physicochemical properties of soil are shown in Table 1.

In each plot, there were 5 rows of 6 m. Plots and blocks were separated by 1 m unplanted distances. Seed were manually sown in individual hills between rows, and intra-row spacing was 50×5 cm. Seed of 'Padideh' safflower were planted on 18th May in 2011, and on 27th May in 2012. For inoculation, seed were coated with gum arabic as an adhesive, and rolled into the suspension of bacteria until uniformly coated. Strain and cell densities of microorganisms used as plant growth-promoting rhizobacteria (PGPR), in this experiment, were 10^7 colony forming units (CFU). Bacteria were isolated from safflower rhizospheres by the Research Institute of Soil and Water, Tehran, Iran. Two seed were sown per hill; and two weeks after the emergence, one plant per hill was thinned at the 4–5 leaf stage. The field was immediately irrigated (by surface irrigation) after planting. Irrigation, weeding, and all other agronomic practices, except for those under study, were kept normal and uniform for all treatments.

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In each experimental plot, two beside rows and a 0.5 m space from the beginning and the ending of the central planting lines were set as margins, and measurements were performed on the three rows in the middle lines. The number of grains per head, number of secondary branches, and the number of grains per plant were determined on 10 randomly selected plants in each plot. Seed oil and fatty acids were extracted according to the Method 988.05 (1990) protocol.

In each sampling, three plants of each plot were taken for the investigation of grain filling parameters. The first samples were taken on the 12th day after flowering, in both years, and the other samples were taken at 4-day intervals for determining the grain weight accumulation. At each sampling, grains were manually removed from the heads and dried at 80°C for 48 hours. Grain dry weight and number were used to calculate the average grain weight for each sample. The total duration of grain filling was determined for each treatment combination by fitting a bilinear model (Borrás & Otegui, 2001):

$$GW = \begin{cases} a + gfr(daa), ...if ... < p_m \\ a + gfr(p_m), ...if ...daa \geq p_m \end{cases}$$

in which: GW is the kernel dry weight; a is the GW intercept; gfr is the slope of grain weight, indicating grain filling rate; daa represents the days after flowering; and p_m is the physiological maturity. Borrás et al. (2004) illustrated grain filling using a bilinear model. Effective grain filling duration (EGFD) was calculated according to Borrás & Otegui (2001), as EGFD = the highest grain weight (g) / ratio of seed filling (g per day).

The kernel weight increase in the filling period was calculated by the above mentioned equation, using the Proc NLIN DUD of the statistical software SAS. Data were statistically analyzed by using SAS, version 9.1 (SAS Institute Inc., Cary, NC, USA). The analysis of variance was used to test the significance;

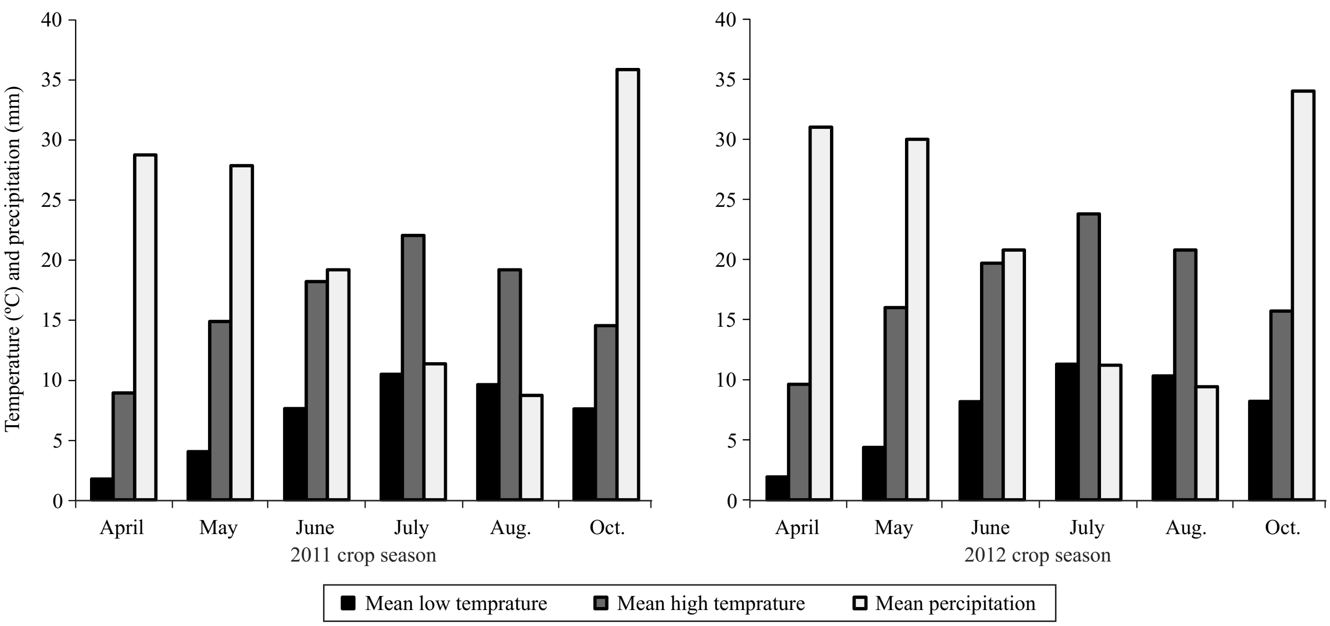

Figure 1. Minimum and maximum temperatures, and precipitation recorded during 2011 and 2012 crop seasons, in Ardabil, Iran.

Table 1. Soil physicochemical properties of the entisol samples at 0–40-cm depth in Ardabil, Iran.

Year	Texture	Sand (g kg-1)	Silt (g kg-1)	Clay (g kg-1)	$CaCO_3$ (%)	Organic carbon (%)	Total N (%)	Exchangeable K (mg kg-1)	Extractable P (mg kg-1)	pH
2011	Silty loam	240	700	50	18.3	0.78	0.16	385	16	8.2
2012	Silty loam	265	680	47	18.0	0.74	0.15	378	16	8.1

and LSD, at 5% probability, was used to compare the means.

Results and Discussion

The number of secondary branches per plant increased about 31.6, 23.0, and 12.2% by the application of 180 kg ha^{-1} urea, in comparison to the application of 0, 60, 120 kg ha^{-1} urea, respectively (Table 2). Further, the lowest and the highest values of this trait were recorded for the treatments with 0 and 180 kg ha^{-1} urea, respectively. Elfadl et al. (2009) and Golzarfar et al. (2012) reported similar results. Moreover, the greatest number of secondary branches per plant were observed in the inoculation treaments. *Pseudomonas* inoculation increased the number of secondary branches by 18.9% in comparison to the noninoculated treatments. These results are in agreement with those reported by Soleymanifard & Sidat (2011) and Mirzakhani et al. (2009) in safflower. The comparison of means showed that the highest number of secondary branches was obtained in the application of 180 kg ha^{-1} urea and seed inoculation with *Pseudomonas*, and the minimum value for this trait was obtained in no inoculation and no N application treatments (Table 3).

The highest number of grains per plant occurred with the 180 kg ha^{-1} urea application, which increased this trait in 36.2% compared to the control in each plant (Table 2). Soleymanifard & Sidat (2011) investigated the effects of N on the growth and yield of safflower, and reported that the number of grains per plant increased as a consequence of N doses up to 30 kg ha^{-1}, but further increase of N doses (30 to 60 kg ha^{-1} N) showed no significant effect on this trait. Biofertilizer inoculations increased the number of grains per plant. Plants treated with inoculation of *Pseudomonas*, *Azospirillum*, and *Azotobacter* showed 9.8, 9.1 and 8.4% more grains per plant than nontreated plants, respectively. Different rates of N fertilizer and inoculation had effects on the number of grains per head. The maximum number of grains per head was observed in the treatment of 180 kg ha^{-1} urea, while the lowest values of this trait was obtained from the control (Table 2). The use of 180, 120, and 60 kg ha^{-1} urea increased the number of grains per head by 27.7, 20.6 and 14.0%, respectively. Dordas & Sioulas (2008) reported that N fertilization increased the number of grains per head by 16% on average. Increasing the number of grains per head may be attributed to the delay of the vegetative and reproductive period, and to the lengthening of grain filling.

Table 2. Effects of biofertilizer (B) and nitrogen (N) rates on grain filling and oil content of safflower (mean of two years, or combined analysis of the two years, 2011–2012) in the studied area, in Ardabil, Iran[(1)].

Treatment	NSB	NGH	Grains per plant	EGFP (day)	GFR (g per day)	GFP (day)	GY (kg ha^{-1})	OC (%)	PA (%)	SA (%)	OA (%)	Lila (%)	Linla (%)
Nitrogen													
N_0 = 0	6.32d	25.28d	137.52d	24.48c	0.00139c	28.43b	1610.5c	25.2c	7.78a	2.48b	13.41c	60.75d	0.056d
N_1 = 60	7.11c	29.66c	181.56c	25.50c	0.00148b	29.45b	1966.0b	26.3b	7.16b	2.44c	13.64c	61.56c	0.061c
N_2 = 120	8.11b	31.86b	200.76b	28.84b	0.00162a	34.49a	2160.1a	27.6a	6.95b	2.56a	14.18b	63.45b	0.0707a
N_3 = 180	9.24a	34.98a	215.76a	30.49a	0.00160a	35.23a	2262.2a	25.5c	6.42c	2.57a	15.30a	64.37a	0.066b
LSD 5%	0.504	1.62	13.56	1.12	0.000431	1.38	114.7	0.428	0.224	0.022	0.336	0.665	0.0025
Biofertilizer													
B_0	7.18b	28.27d	171.00b	25.75d	0.00149b	30.12d	1930.2c	24.9c	8.25a	2.70a	12.90d	60.16d	0.052d
B_1	8.86a	31.92a	189.62a	28.97a	0.00170a	33.64a	2075.8a	26.4a	6.25d	2.51b	14.47a	64.37a	0.069a
B_2	7.39b	30.30c	188.28a	27.96b	0.00149b	31.24c	1979.4b	25.9b	6.64c	2.68a	13.95b	62.29b	0.650b
B_3	7.35b	31.27b	186.72a	26.69c	0.00151b	32.60b	1989.5b	25.9b	7.11b	2.68a	13.64c	61.23c	0.058c
LSD 5%	0.313	0.636	3.47	0.56	0.0004	0.61	91.3	0.251	0.345	0.036	0.263	0.717	0.0025
N	*	*	*	**	**	**	*	*	*	*	**	*	*
B	*	**	**	**	**	**	**	*	**	*	*	*	**
N × B	*	*	**	**	**	**	*	**	*	ns	ns	**	ns

[ns]Nonsignificant. *, **Significant differences at 5% and 1% probability, respectively. NSB, number of secondary branches; NGH, number of grains per head; EGFP, effective grain filling period; GFR, grain filling rate; GFP, grain filling period; GY, grain yield; OC, oil content; PA, palmitic acid; SA, stearic acid; OA, oleic acid; Lila, linoleic acid; Linla, linolenic acid. N_0, no nitrogen application; N_1, N_2, and N_3, application of 60, 120, and 180 kg ha^{-1} urea, respectively. B_0, no seed inoculation; B_1, B_2, and B_3, seed inoculation by *Pseudomonas putida* strain 186, *Azospirillum lipoferum* strain F, and *Azotobacter chroococcum* strain 5, respectively.

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Application of high N rates (180 kg ha^{-1} urea) and seed inoculation by *Pseudomonas* resulted in 25.66% increase of the grain filling potential rate, in comparison to the control (Table 2). In other words, peak grain filling rates lasted longer in the treatment with high N rates and seed inoculation with *Pseudomonas* than the control treatment (Figure 2 D). Borrás et al. (2004) reported that the lack of assimilate supply, could result in a dramatic decline in grain filling period and grain weight. Massignam et al. (2009) noted that N plays an imperative role in the maximization of crop yields, via its effects on photosynthetic processes such as grain filling rate and grain filling period. Abbadi et al. (2008) showed that increasing N fertilizer rates increased traits related to grain growth in safflower. Moreover, Hamidi et al. (2009) stated that grain filling period was prolonged due to inoculation by PGPR in maize hybrids. They suggested that inoculation by PGPR by various mechanisms – such as the ability to produce indoleacetic acid, gibberelic acid, cytokines, symbiotic N fixation, and antagonism to phytopathogenic microorganisms by the production of siderophores – causes the increase of grain filling period. High grain filling rate by the application of 180 kg ha^{-1} urea and seed inoculation by *Pseudomonas* could result from sufficient assimilate supply and large partitioning capacity.

Grain filling duration was delayed by seed inoculation, high rates of N application, and their combinations as compared to the control. The maximum grain filling duration was observed in the treatment with the highest N rate (Table 2). Among the biofertilizer treatments, seed inoculation by *Pseudomonas* increased grain filling duration more than *Azotobacter* and *Azospirillum*. Grain filling duration increased in plots that received the highest rate of N application and seed inoculation by *Pseudomonas* (Table 3). Similar results were obtained in effective grain filling period. Hamidi et al. (2009) reported that high N rates significantly delay the duration of the vegetative and reproductive periods, and could be the possible reason for lengthening of grain filling duration.

Effects of N rates on fatty acid compositions were significant (Table 2). Linoleic acid ($C_{18:2}$) was the most abundant fatty acid, ranging between 60.75–64.37%, followed by oleic acid ($C_{18:1}$, 13.41–15.3%) and palmitic acid ($C_{16:0}$, 6.95–7.78%), by the application of 0 and 180 kg ha^{-1} urea respectively. The amount ranges of linolenic acid ($C_{18:3}$) and stearic acid ($C_{18:1}$) were

Table 3. Effects of biofertilizer (B) and N rates on grain filling and oil content of safflower (mean of two years, or combined analysis of the two years, 2011–2012) in the studied area, Ardabil, Iran[(1)].

Treatment compound	NSB	Grains per plant	GFR (g per day)	EGFP (day)	GFP (day)	Fitted equations	NGH	OC (%)	PA (%)	Linla (%)	GY (kg ha^{-1})
N_0 B_0	5.56h	118.4i	0.00139f	23.03j	26.71k	y=-0.00972+0.00146x	21.26i	27.9i	7.78a	60.75i	1515.3i
N_0 B_1	7.32ef	152.7g	0.00157c	25.93f	29.98gh	y=-0.013+0.0016x	28.38g	31.4def	7.16c	64.37c	2238.3bc
N_0 B_2	6.14g	140.8h	0.00149d	24.81g	28.95hi	y=-0.00997+0.00147x	25.48h	31.16fg	6.42f	63.45de	2130.9d
N_0 B_3	6.28g	142.1h	0.00145de	23.71ji	27.7kj	y=-0.00976+0.0015x	26.45h	30.92g	6.25f	61.56h	1943.8f
N_1 B_0	6.52g	159.2g	0.00144e	23.94hi	27.0k	y=-0.00647+0.0014x	28.02g	31.28efg	7.57b	60.70i	1605.2h
N_1 B_1	7.73de	191.7f	0.00159bc	26.31ef	31.39f	y=-0.0107+0.00163x	30.29f	32.85cd	6.95d	65.14b	2249.6bc
N_1 B_2	7.00f	191.7f	0.00149d	26.31ef	30.19g	y=-0.00785+0.00146x	30.07f	31.77cd	6.32f	63.62de	2174.4cd
N_1 B_3	7.00f	188.0f	0.00145de	24.71gh	28.6ji	y=-0.00967+0.0015x	30.68ef	31.52def	6.27f	62.24g	1959.3ef
N_2 B_0	7.97d	195.3ef	0.00139f	27.1de	32.59e	y=-0.0064+0.0014x	29.95f	31.64cde	5.80g	61.38h	1735.4g
N_2 B_1	9.18b	203.5de	0.00159bc	30.19ab	35.68b	y=-0.0099+0.00168x	33.09cd	33.34a	5.95g	66.61a	2278.9ab
N_2 B_2	7.73de	206.6cd	0.00158c	29.4b	34.88bc	y=-0.00803+0.00148x	31.89de	32.01c	6.26f	63.89cd	2190.1cd
N_2 B_3	7.61de	203.1de	0.00147cd	28.2c	33.78cd	y=-0.0102+0.0016x	33.09cd	32.49b	6.72e	62.62fg	1988.8ef
N_3 B_0	8.56c	207.9bcd	0.00158c	27.9cd	33.58de	y=-0.00881+0.00164x	34.3bc	29.35h	6.95d	62.24g	1788.3g
N_3 B_1	11.35a	219.0a	0.00187a	30.79a	36.87a	y=-0.0121+0.00191x	36.6a	31.64cde	5.83g	65.36b	2344.0a
N_3 B_2	8.69c	217.6ab	0.00163b	29.7b	35.48b	y=-0.0104+0.00165x	34.3bc	31.52def	6.25f	64.44c	2237.4bc
N_3 B_3	8.51c	215.4abc	0.00159bc	28.5c	34.28cd	y=-0.00971+0.00169x	35.51ab	31.77cd	6.25f	63.14ef	2024.4e
LSD	0.438	9.92	0.000394	0.81	1.11	-	1.27	0.4031	0.196	0.572	79.45

[(1)]Means with equal letters, in the columns, are not different by LSD test at 5% of probability. N_0, no nitrogen application; N_1, N_2, and N_3, application of 60, 120, and 180 kg ha^{-1} urea, respectively. B_0, no seed inoculation; B_1, B_2, and B_3, seed inoculation by *Pseudomonas putida* strain 186, *Azospirillum lipoferum* strain F, and *Azotobacter chroococcum* strain 5, respectively. NSB, number of secondary branches; GFR, grain filling rate; EGFP, effective grain filling period; GFP, grain filling period; NGH, number of grains per head; OC, oil content; PA, palmitic acid; Linla, linolenic acid; GY, grain yield.

0.056-0.0707% and 2.48-2.57%, respectively; these ranges are similar to those reported by Coşge et al. (2007) in safflower. The application of 120 kg ha^{-1} urea increased the linoleic acid content in 5.6, 4.36, and 1.4%, in comparison to the applications of 0, 60, and 180 kg ha^{-1} urea, respectively. Safflower oil composition determines the oil quality, and the oil fatty acid composition varies according to the environmental conditions during grain filling. *Pseudomonas* inoculation induced 6.5% increase of linoleic acid content (Table 2). The saturated fatty acids (palmitic and stearic acids) reduced in the treatment with *Pseudomonas* inoculation, in comparison to the control, while unsaturated fatty acids (linoleic, linolenic, and oleic acids) increased. Silva et al. (2013) reported that the biofertilizer inoculation enhances fatty acids content of soybean seed. The comparison of means showed that both inoculation and N application induced a content increase of linoleic acid. The treatments with application of 60, 120, and 180 kg ha^{-1} urea and the *Pseudomonas* inoculation showed the highest linoleic fatty acid content – 6.73, 8.1, and 7% increase, respectively, in comparison to the control. Conversely, these treatments resulted in the lowest content of palmitic acid, with 10.8, 23.0, and 25.5% decrease, respectively. Silva et al. (2013) reported that the biofertilizer inoculation enhances unsaturated fatty acids content of soybean seed. Similar results have been reported by Coşge et al. (2007) in safflower.

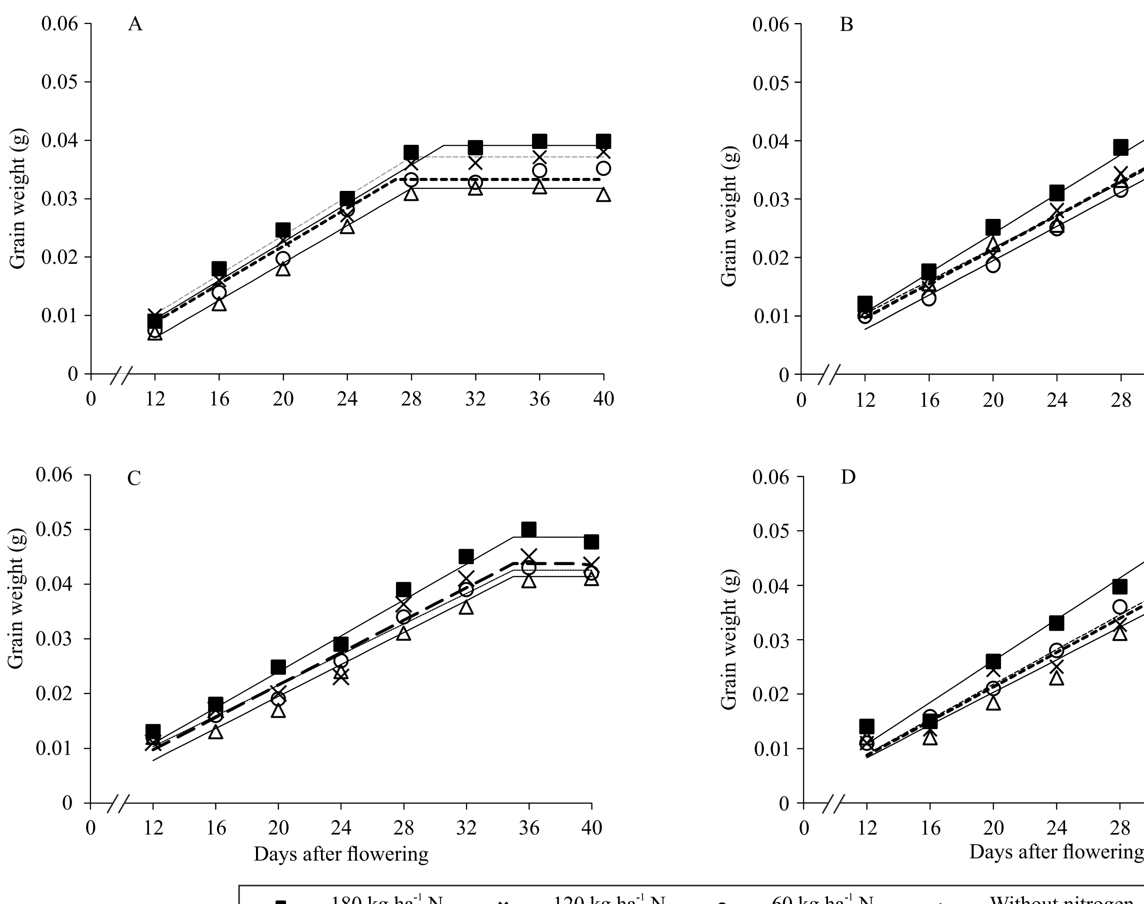

Figure 2. Variation of grain weight and of grain filling period in safflower plants subjected to N application at various rates, and seed inoculation by biofertilizers. A, no seed inoculation. Seed inoculation: B, by *Azotobacter chroococcum* strain 5; C, by *Azospirillum lipoferum* strain F; and D, by *Pseudomonas putida* strain 186. Mean of two years, or combined analysis of the two years (2011–2012) in the studied area, in Ardabil, Iran.

Oil content is one of the more important components, which play a crucial role, in safflower seed quality. A slight increase of oil content was observed by increasing N rates up to 120 kg ha^{-1} urea; however, by applying the highest N level, the oil content decreased again, which agrees with Abbadi et al. (2008), who found that at high N levels there was a significant decrease of oil content. However, Dordas & Sioulas (2008) found no relationship between N rates and oil content. In the present study, the maximum seed oil content was obtained by applying 120 kg ha^{-1} urea and *Pseudomonas* inoculation; minimum seed oil content was observed in the control treatment (Table 3). The seed oil content increased by 8.7% with increasing N applications from 0 to 120 kg ha^{-1} urea, and, then, it decreased significantly. Golzarfar et al. (2012) found that increasing N rates from 0 to 150 kg ha^{-1} urea increased the means of all traits, except for seed oil content which had a slight decrease in the highest level of N. Biofertilizer inoculated plants showed a higher seed oil content than the control plants (Table 2). *Pseudomonas* inoculation induced 5.6% increase of oil content, in comparison to the control treatment. Both inoculation and N application induced an increase of oil content. The treatments with 120 kg ha^{-1} urea and seed inoculation by *Pseudomonas* showed the highest oil content (16.3% increase). Shehata & El-Khawas (2003) reported that seed treated with PGPR inoculation increased oil content of sunflower. Silva et al. (2013), in a soybean study, and Coşge et al. (2007), in a safflower study, reported similar results.

The different combinations of seed treated with biofertilizer inoculation and N-fertilization treatments had effect on the grain yield of safflower (Table 3). The highest grain yield was obtained in the integrated treatment of 180 kg ha^{-1} urea and *Pseudomonas* inoculation, which had a significant difference in comparison to other integrated treatments. The minimum grain yield was obtained in the control treatment. Biofertilizer applications showed a promoting effect on the grain yield, comparing with the uninoculated treatments. The biofertilizer treatment with *Pseudomonas*, *Azotobacter*, and *Azospirillum* increased grain yield by 6.97, 2.5, and 3.0%, respectively, in comparison to the control. Similar findings were also reported by Shoghi-Kalkhoran et al. (2013), who stated that biofertilizer alone, or in combination with synthetic fertilizers, significantly increased grain. Stimulation of different crops by biofertilizer inoculations has also been shown by other studies, both in laboratory and field trials. According to Stefan et al. (2013), yield increased up to 18.42% with *Bacillus pummilus* inoculation, and up to 33.36% with *Bacillus mycoides* inoculants. Soleymanifard & Siadat (2011) have been shown that safflower yield increased by the seed treatment with PGPR inoculation.

Conclusions

1. Biofertilizer inoculation and N application have effects on the grain filling period, and on the quantitative and qualitative yield of safflower.
2. Contents of saturated fatty acids reduce as a consequence of seed treated with biofertilizer inoculations, while unsaturated fatty acids increase.
3. The suitable amount of N fertilizer application (between 120 and 180 kg ha^{-1} urea) can improve plant growth and the quantity and quality of oil, in seed treated with *Pseudomonas putida* inoculation, in safflower plants.

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