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ROLE OF RHIZOBACTERIA INOCULATIONS ON AGRONOMIC AND QUALITY CHARACTERISTICS OF SAFFLOWER (*Carthamus tinctorius* L.) UNDER UNFERTILIZED CONDITIONS

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ABSTRACT

Safflower is an important, but ignored oil crop species in Turkiye, and it is necessary to increase yield to increase the cultivation of the crop. The present study was carried out to determine the effects of rhizobacteria seed inoculation on agronomic characteristics and fatty acid contents of safflower cultivars in 2019-2020 croping seasons in Isparta. Seeds of Dincer 5-18-1, Linas and Montola 2000 were inoculated with *Bacillus* species before sowing. Results revealed that year, cultivar and applications significantly affected 1000-seed weight, seed and oil yields. Seed weights of the rhizobacteria inoculated cultivars increased significantly and was higher (38.5 g) than the uninoculated seeds (36.3 g). Seed yields were higher for both years for rhizobacteria inoculation. Seed and oil yield increased in rhizobacteria inoculated seeds by 20% and 18%, and reached to 128.8 and 34.0 kg da^{-1}, respectively compared to the uninoculated seeds (107.2 and 28.6 kg da^{-1}). Seed inoculations increased linoleic acid and decreased oleic acid contents in Dincer 5-18-1 and Linas. Seed inoculations with rhizobacteria could be used as an easy, practical and efficient approach to improve plant growth, seed and oil yields of safflower under field conditions.

Keywords: ***Bacillus*, bacteria, fatty acid, safflower, seed, yield**

INTRODUCTION

Safflower (*Carthamus tinctorius* L.) is an important oil seed crop that originated from Mediterranean basin, and it has been cultivated since antiquity for its seeds and flowers with the aim of oil and dye production (Knowles, 1989). Even though safflower genotypes with high oil content have been identified (Johnson et al., 1999), most of the safflower cultivars contain 26-34% oil (Erbas et al., 2016). The cultivated area (24.693 ha) and the total production (35 tons) reached the highest levels in Turkiye, however, in the last three years, both total cultivated area and total production decreased (TUIK, 2021). Production of oil seed crops domestically does not meet the demand and there is a growing shortage for edible oils. To meet increasing demand for cooking oil, oil seeds and raw oil were imported with a value of over 4 billion dollars in 2021 (TUIK, 2021).

Safflower is grown mainly in arid and semi-arid regions with the ability of drought tolerance due to its deep root system. Safflower yields varied from 60 to 193 kg da^{-1} between 2000-2021 with average yield of 123 kg da^{-1} (TUIK, 2021). Although safflower is a valuable oil seed crop, its seed yield is low compared to other oil seed crops, such as canola and soybean, which prevents the spread of safflower cultivation to higher levels in Turkiye and in the world. The yield of all cultivated plants depends on the genetic potential, cultural practices, environmental conditions and their interactions. Through manipulation of cultural practices, such as weed and disease control, early sowing, fertilization and seed treatments, it is possible to increase yields of the crops. It has been reported that earlier sowing (Yau, 2007; Keles and Ozturk, 2012; Hatipoglu et al., 2012) and application of different fertilizers (Tuncturk and Yildirim, 2004; Katar et al., 2012; Ekin, 2020) increased seed and oil yield in safflower. Different seed treatments including inoculation with plant growth promoting bacteria, have also been used to increase germination, seedling establishment, and crop productivity and quality under normal or stress conditions in many different crop species including safflower (Naseri and Mirzaei, 2010; Nosheen et al., 2016; Nosheen et al., 2018a; Khademian et al., 2019; Zhang et al., 2019).

Different bacteria that live in soil and colonize rhizosphere of plant roots have beneficial roles on nutrient cycling, crop production, soil health and biota, and referred commonly as plant growth promoting bacteria (PGPB) or rhizobacteria encompassing many different bacteria species, such as *Pseudomonas*, *Bacillus*, *Enterobacter*, *Azotobacter*, *Azosprillum* spp. (Ramakrishna et al., 2019). Rhizobacteria use has gained great attention due to their roles in nitrogen fixation, phosphate solubilization, nutrient uptake, protection against soil borne diseases and stimulating phytohormone production, all of which help to improve crop productivity under field conditions. Remobilization and accumulation of nutrients in soil help to support sustainable agriculture practices by reducing fertilizer and pesticide dependence for yield increases in crop species (Ozturk and Yildirim, 2013; Mahmood et al., 2016). Rhizobacteria could be applied through seed inoculation, spraying and soil broadcasting (Ashraf and Foolad, 2005; Aslantas et al., 2007) depending on plants and inoculation conditions, but seed inoculation is the most used approach due to its practicality (Mahmood et al., 2016). Safflower yields increased with co-application of rhizobacteria and different chemical and organic fertilizers (Naseri and Mirzaei, 2010; Sharifi et al., 2017; Nosheen et al., 2018a; Ekin 2020; Heidari et al., 2020), seedling emergence, growth and root development (Nosheen et al., 2011; Zhang et al., 2019), seed quality and mineral content (Ekin, 2020; Heidari et al., 2020; Nosheen et al., 2016), but all of these reports used rhizobacteria and different forms of fertilizers and doses. Some of these researchers have also noted that amount of fertilizers could be reduced from the recommended doses with the use of rhizobacteria (Naseri and Mirzaei, 2010; Nosheen et al., 2018a). Thus, rhizobacteria applications could be an environmentally friendly and inexpensive alternative to use of fertilizers to increase nutrient availability in soil for crop production (Cakmakci et al., 2001; Cakmakci et al., 2006). Therefore, the aim of the present research was to evaluate effects of seed inoculation with rhizobacteria on yield and yield components of safflower without fertilizer applications under ecological conditions of Isparta province.

MATERIALS AND METHODS

Three safflower cultivars (Dincer 5-18-1, Linas and Montola 2000) were used as plant material in the study. Oil content and 1000-seed weight of the safflower cultivars are between 29.0-35.1% and 38.8-40.4 g, respectively (Erbas et al., 2016; Senates and Erbas, 2020). The field research was conducted at the research farm of Isparta University of Applied Sciences in Isparta (37° 45´ N, 30° 33´ E, elevation 997 m), which is located in inner Mediterranean Region, during 2019 and 2020 crop seasons. Monthly mean temperatures and total precipitation for 2019 and 2020 along with long term climatic data of the research area were given in Table 1. Extremely high or low precipitation and temperature fluctuations did not occur during the vegetation period (March–August) that would severely affect the growth and development of the plants. In 2019, 190.8 mm of total precipitation recorded from sowing to seed development period, while recorded precipitation was 226.9 mm for the same period in 2020. However, total precipitation was higher during the flowering period in 2020 (92.1 mm) than in 2019 (34.2 mm). During the seed development and maturing period, higher than average temperatures were recorded in 2020.

Table 1. Long term (1929-2021) and monthly mean temperature (°C) and monthly total rainfall (mm) for 2019-2020 of Isparta province.

Months	Temperature (°C)			Precipitation (mm)		
	2019	2020	1929 – 2020	2019	2020	1929 – 2020
January	2.5	1.4	1.8	97.0	74.1	81.0
February	4.5	3.8	3.0	55.4	71.4	67.0
March	7.3	7.7	6.0	40.3	41.3	58.7
April	9.9	11.6	10.7	50.8	24.2	51.6
May	16.8	16.1	15.5	34.2	92.1	56.4
June	20.7	20.3	19.9	53.3	42.6	35.5
July	23.3	27.0	23.4	9.5	1.9	15.8
August	24.4	25.6	23.3	2.7	24.8	14.1
September	20.0	24.1	18.9	26.5	1.0	18.5
October	15.7	17.4	13.4	9.9	48.7	38.1
November	9.8	9.3	7.9	28.6	26.5	44.6
December	4.6	6.9	3.6	45.3	35.4	87.1
Total	–	–	–	453.5	484.0	568.4
Mean	13.3	14.3	12.3	–	–	–

The soil physical and chemical properties of the research area was analyzed following Rowell (1996). The soil was clay-loam, total lime was 7.1% (Schiebler calcimeter method), total nitrogen content was 0.18% (macro Kjehldal method), exchangeable K and P contents were 137 mg kg^{-1} and 10.6 mg kg^{-1}, respectively (Olsen method). Soil organic content was 1.2% (Walkley-Black method) with a pH value of 7.8.

Commercially available rhizobacteria solution (LIFEBAC-NP) was purchased from Bactogen Inovatif Tarım (Istanbul, Turkiye) and the commercial solution contained *Bacillus subtilis* ($1x10^9$ cfu mL^{-1}) and *B. megaterium* ($1x10^8$ cfu mL^{-1}). These *Bacillus* spp. were shown to have nitrogen fixing and phosphate solubilizing abilities and acted as plant bio-stimulants and bio-fertilizers (Cakmakci et al., 2001; Cakmakci et al., 2006). Seeds of the safflower cultivars were inoculated either with liquid bacterial solution as recommended or with distilled water for 60 min, and the seeds allowed to dry at room temperature before sowing.

The experiment was set up as randomized complete block design with three replications. Seeds were sown on March 27 and April 1 in 2019 and 2020, respectively. Plot length was set to 5 m and each plot contained 5 rows. Spacing between rows was 0.5 m and within rows was 0.15 m (Erbas et al., 2016). Plots did not receive any fertilization or irrigation and weed control after emergence was done by manual and mechanical hoeing. Three middle rows were harvested at the last week of September in both years. Ten plants were used from each replication to measure following parameters; plant height (cm), number of branches per plant, capitulum number per plant, capitulum diameter (mm), stem diameter (mm), 1000-seed weight (g), harvest index (%), seed yield (kg da^{-1}), hull content (%), oil content (%), oil yield (kg da^{-1}) and fatty acids composition (%). The oil content and fatty acid compositions of the cultivars between the applications were determined as described by Erbas et al. (2016). Results were subjected to analysis of variance (ANOVA) using IBM SPSS Statistics 22.0 software (SPSS Inc., Chicago, IL, USA) and the differences between the means were separated by Duncan's multiple range tests (Dowdy and Wearden, 1983).

RESULTS AND DISCUSSION

The results of ANOVA analysis revealed that growth year was significant for all parameters ($p \leq 0.01$) except the hull content (Table 2). Capitulum and stem diameters were not statistically different from each other between the cultivars. Rhizobacteria inoculation showed significant effects on 1000-seed weight, seed and oil yields. Interactions between year × cultivar, year × application, cultivar × application revealed some significant effects as well. Number of branches and capitulum number per plant were affected by year × cultivar interaction, capitulum number per plant, capitulum diameter and stem diameter were affected by year × application interaction and number of branches per plant, capitulum number, harvest index and seed yield were affected by cultivar × application interaction. Year × cultivar × application interaction was only found to be significant for number of branches per plant, and other important interactions were also shown in Table 2 separately.

Plant height, number of branches and capitulum number per plant were not significant for the rhizobacteria inoculation, but the differences between these parameters were significant between the cultivars and between the years (Table 3). Average plant height, number of branches and capitulum number were higher in the first year of the study. Among the cultivars, Linas had the higher plant height and number of branches, but had the lowest capitulum number for per plant. Different results for the rhizobacteria inoculations on plant height, number of branches and capitulum number were reported. While Naserzadeh et al. (2018) did not report significant differences for plant height and branch number inoculations with *Pseudomonas* spp.; different rhizobacteria applications increased shoot and root lengths (Ekin, 2020; Nosheen et al., 2018a), number of branches (Ekin, 2020) and capitulum number per plant (Ekin, 2020; Naseri and Mirzaei, 2010) in safflower. However, number of flowers was not affected by rhizobacteria application as reported by Naserzadeh et al. (2018). Capitulum number per plant is low and plant height and number of branches are moderately heritable characters (Kotecha, 1981; Reddy et al., 2004; Camas and Esendal, 2006), therefore they could be affected by the environmental conditions (Camas et al., 2007). Even though there were no differences for plant height, capitulum number and number of branches among the applications, they were different between the years and between the cultivars showing that expression of these characters change depending on years and genotypes (Erbas et al., 2016; Senates and Erbas, 2020).

Capitulum diameter has a low inheritance hence the differences observed for capitulum diameter should come from growth conditions (Camas and Esendal, 2006). Capitulum diameter could be between 10-35 mm (Weiss, 2000) and our results showed that rhizobacteria inoculations did not cause any significant difference in capitulum and stem diameters between the cultivars and the applications (Table 3 and 4). However, capitulum diameter was higher in the second year and stem diameter was higher in the first year of the study (Table 3). Previous reports have also noted that capitulum and stem diameters could be different between the years and between the genotypes (Erbas and Tonguc, 2009; Erbas et al., 2016). Even though rhizobacteria application increased average capitulum and stem diameter in the present study, these were not statistically significant as was noted by Naserzadeh et al. (2018), but significant increases for these characters were reported for safflower grown in Van ecological conditions (Ekin, 2020).

Hull content of the cultivars varied significantly, but it was not significantly affected by rhizobacteria application and years, though average hull content slightly reduced in rhizobacteria applied cultivars (Table 4). Dincer 5-18-1 had higher hull content than the other cultivars. Hull content reduces both oil and protein contents (Dajue and Mundel, 1996), and it is controlled by major genes, which make hull content less sensitive to growth conditions (Ebert and Knowles, 1966), as was noted in the present study. Similarly, rhizobacteria applications did not change significantly seed/kernel ratios in safflower (Naserzadeh et al., 2018).

Harvest index did not show significant differences between the applications, but rhizobacteria applications had slightly higher harvest index values (Table 4). There was a significant difference between the years, and the harvest index of 2020 was higher than the harvest index of 2019.

Table 2. Results of analysis of variance for agronomic characteristics of safflower cultivars.

Sources of variance	df	Plant height	Branch number	Capitulum number	Capitulum diameter	Stem diameter	Hull content	1000 seed weight	Ha i
Replication	2	10.2[a]	0.7	5.3	2.2	0.3	26.1	1.4	
Years (Y)	1	769.1**	9.1**	192.7**	126.2**	18.2**	0.4	27.2**	6
Cultivars (C)	2	121.9**	8.6**	24.1*	0.1	0.3	144.7**	5.7**	13
Application (A)	1	1.3	0.03	0.02	0.6	0.09	1.0	44.7**	
Y x C	2	13.2	1.9*	20.0*	4.4	0.8	33.4	0.3	
Y x A	1	22.1	0.2	22.9*	12.5**	6.2**	32.9	0.06	0
C x A	2	7.7	7.3**	21.7*	0.05	0.5	2.2	1.5	1
Y x C x A	2	3.7	2.5*	5.3	0.6	0.1	0.1	0.1	0
Error	22	6.4	0.5	93.6	31.9	13.7	219.9	18.3	
CV (%)		4.0	7.4	11.9	5.5	8.4	6.3	2.4	

a, mean squares of characters; df, degree of freedom; CV, coefficient of variation; *$p \leq 0.05$; **$p \leq 0.01$

Table 3. Means of seed treatments with rhizobacteria on plant height, branch number, capitulum number and capitulum dam

	Year	Plant height (cm)			Branch number			Capitulum number		
		Control	RI	Mean	Control	RI	Mean	Control	RI	Mea
	2019	67.1	69.1	**68.1 A**	9.6	9.6	**9.6 A**	18.9	20.5	**19.7**
	2020	59.5	58.3	**58.9 B**	8.7	8.5	**8.6 B**	15.8	14.3	**15.1**
Cultivars	Dincer 5-18-1	62.5	61.7	**62.1 B**	7.4	9.1	**8.2 C**	16.3	18.5	**17.4 A**
	Montola 2000	60.1	62.3	**61.2 B**	9.5	8.8	**9.2 B**	20.3	17.3	**18.8**
	Linas	67.3	67.0	**67.1 A**	10.5	9.3	**9.9 A**	15.5	16.5	**16.0**
Cultivar Mean		**63.3**	**63.7**		**9.1**	**9.1**		**17.4**	**17.4**	

RI, rhizobacteria inoculation; Different letters indicate significant differences at $p \leq 0.05$

Among the cultivars, Dincer 5-18-1 had higher harvest index value than the Linas and Montola 2000. Similarly, rhizobacteria inoculations increased harvest index but it was not significant between the applications and control in safflower (Naserzadeh et al., 2018). Increased capitulum diameter and 1000-seed weight and decreased plant height in the second year of the study could contribute higher index values observed in 2020. Harvest index values ranged from 8-30% among 39 cultivars (Erbas et al., 2016) and Dincer 5-18-1 had higher harvest index than the other cultivars in the study, and harvest index could pass 40% among the diverse safflower genotypes (Erbas and Tonguc, 2009). Irregular rainfall in April and June 2020, the vegetative development period of safflower, may have caused the lower plant height, number of branches, number of heads observed for the same year. Harvest index was lower in 2020 as well, which might result from lower seed yield due to lower plant height, number of branches, number of heads observed for the same year.

Seed weights of rhizobia inoculated cultivars were higher in both years, and as a result, combined years analysis showed that rhizobacteria inoculation increased 1000-seed weight of the cultivars (Table 4). In addition, 1000-seed weights of the cultivars was higher in the second year of the study. Among the cultivars, Dincer 5-18-1 had higher 1000-seed weight than Montolo 2000. One thousand seed weights of these cultivars were between 36-40 g in Isparta (Erbas et al., 2016; Senates and Erbas, 2020) and our current results fell into the reported ranges. Rhizobacteria applications increased 1000-seed weight of safflower (Naserzadeh et al., 2018; Ekin, 2020), but the increase was more pronounced when it was used with the fertilizers (Naseri and Mirzaei, 2010; Ekin, 2020; Heidari et al., 2020).

Seed yield changed between 102.1-126.3 kg da^{-1} for the cultivars, and Linas and Dincer 5-18-1 were higher yielding varieties based on average of two years. Seed yields in first year of the research was lower than the second year. Inoculation of safflower seeds with rhizobacteria significantly increased seed yields as well (Table 5). While control application average seed yield was 107.2 kg da^{-1}, rhizobacteria inoculation increased seed yield to 128.8 kg da^{-1}. Seed yields of rhizobacteria inoculated cultivars were higher than control at both years as well. Safflower yields varied between 60-193 kg da^{-1} with average yield of 123 kg da^{-1} in Turkiye for the last 10 years (TUIK, 2021). Though lower and higher yields for safflower have been reported, such as 207.7-339.7 kg da^{-1} in Eskisehir (Celikoglu, 2004), 24.2-180.5 kg da^{-1} for cultivars and 53.6-277.5 kg da^{-1} for plant introductions in Isparta (Erbas and Tonguc, 2009; Erbas et al., 2016), 45.6-298.0 kg da^{-1} in Samsun (Camas and Esendal, 2006), 163.7-262.3 kg da^{-1} in Mardin (Arslan and Guler, 2022). Rhizobacteria applications or in combination with different types of fertilizers increased seed yields of safflower. Naseri and Mirzaei (2010) and Khademian et al. (2019) obtained 80.1 and 293.1 kg da^{-1} yields from control, but with application of *Azotobacter* and *Azospirillum*, seed yields increased to 123.5 and 102.1 kg da^{-1}, and 320.0 and 337.3 kg da^{-1}, respectively. Ekin (2020) reported that yield increased from 140 kg da^{-1} to 206 kg da^{-1} with the use of *Bacillus* spp., and when bacteria inoculations were supplemented with humic acid doses, yield of Dincer 5-18-1 increased up to 232 kg da^{-1}. Co-application of different forms and doses of fertilizers with rhizobacteria yielded significant increases. Heidari et al. (2020) noted that rhizobacteria inoculations with different biochar fertilizers increased seed yields. The highest increase was observed from manure biochar supplemented with phosphate solubilizing microorganisms. Rhizobacteria applications increase soil microbial and enzyme activities, which in turn increase plant root development and root area, chlorophyll content (Nosheen et al., 2011; Nosheen et al., 2018a; Heidari et al., 2020). Seed yield has low heritability and seed yields could be affected by precipitation, temperature, seed treatments, fertilization and nutrient availability (Reddy et al., 2004; Camas and Esendal, 2006; Camas et al., 2007). When seed yield was used as the dependent variable in path analysis, plant height, number of capitulum, stem diameter, capitulum diameter, duration of flowering period and 1000-seed weight had direct positive effects, and the number of branches per plant had negative effects on yield (Bidgoli et al., 2006; Arslan, 2007). Our results show that rhizobacteria inoculations stimulated plant growth and increased 1000-seed weight, thus increased the seed yields at both years of the research.

Rhizobacteria inoculations did not cause an increase in oil content. However, oil content between the years was different and the second year of the research had significantly higher oil yields (Table 5). Among the cultivars, Linas (28.7%) had the highest oil content followed by Montola 2000 (26.9%) and Dincer 5-18-1 (24.1%). Results of the present research was similar to previous results for the same cultivars (Erbas et al., 2016; Senates and Erbas, 2020). Effects of rhizobacteria inoculations on oil content differs depending on microorganisms used. While *P. florescence* strains did not significantly increase the oil content, some *P. putida* strains increased the oil content (Naserzadeh et al., 2018). Similarly, *Azotobacter* and *Azospirillum* inoculations increased the oil content of safflower seeds by alone or in combination with supplemental fertilizer applications (Naseri and Mirzaei, 2010; Khademian et al., 2019).

Oil yields of the cultivars were higher for rhizobacteria inoculated seeds for both years and oil yield between the applications differed significantly and rhizobacteria inoculated seeds (34.0 kg da^{-1}) possessed higher oil yield than the untreated seeds (28.6 kg da^{-1}) per decare. Safflower cultivars also differed for their oil yields and Linas had the highest oil yield in the study followed by Dincer 5-18-1 and Montola 2000 (Table 5). Difference between the years was also significant and the second year of the experiment gave higher oil yields. Result of oil yield was different in the current study than our previous study where Linas had the lowest oil yield among the cultivars (Senates and Erbas, 2020). Significant oil yield increases as a result of rhizobacteria inoculation was also reported for different bacteria species (Khademina et al., 2019; Ekin, 2020).

Table 4. Means of stem diameter, hull content, 1000-seed weight and harvest index of safflower cultivars treated v

Year	Stem diameter (mm)			Hull content (%)			1000-seed weig	
	Control	RI	Mean	Control	RI	Mean	Control	RI
2019	9.7	10.6	**10.2 A**	49.4	51.0	**50.2**	35.4	37.7
2020	9.1	8.4	**8.7 B**	51.1	48.8	**50.0**	37.2	39.3
Cultivars: Dincer 5-18-1	9.3	9.9	**9.6**	54.5	53.2	**53.9 A**	37.4	38.8
Montola 2000	9.5	9.2	**9.3**	49.1	49.5	**49.3 B**	35.4	38.1
Linas	9.4	9.4	**9.3**	47.1	47.0	**47.0 B**	36.0	38.7
Cultivar Mean	**9.4**	**9.5**		**50.2**	**49.9**		**36.3 B**	**38.5 A**

RI, rhizobacteria inoculation; Different letters indicate significant differences at $p \leq 0.05$

Table 5. Means of seed yield, oil content and oil yield of safflower cultivars treated with rhizobacte

Year	Seed yield (kg da^{-1})			Oil content (%)		
	Control	RI	Mean	Control	RI	Mean
2019	101.5	122.0	**111.8 B**	25.2	25.7	**25.4 B**
2020	112.8	135.6	**124.2 A**	27.6	27.7	**27.6 A**
Cultivars: Dincer 5-18-1	113.5	137.6	**125.5 A**	24.1	24.2	**24.1 C**
Montola 2000	95.1	109.2	**102.1 B**	26.9	26.8	**26.9 B**
Linas	113.0	139.7	**126.3 A**	28.2	29.2	**28.7 A**
Cultivar Mean	**107.2 B**	**128.8 A**		**26.4**	**26.7**	

RI, rhizobacteria inoculation; Different letters indicate significant differences at $p \leq 0.05$

Table 6. Fatty acid compositions of safflower cultivars grown in 2019 and 2020.

Fatty acids	2019							
	Dincer 5-18-1		Montola 2000		Linas		Dincer 5-18-1	
	Control	RI	Control	RI	Control	RI	Control	RI
Palmitic acid ($C_{16:0}$)	6.5	6.3	6.6	6.3	6.5	6.6	7.1	5.9
Stearic acid ($C_{18:0}$)	2.2	1.8	2.1	2.0	2.1	2.2	2.7	2.0
Oleic acid ($C_{18:1}$)	10.8	10.2	76.5	75.2	26.0	14.8	10.6	10.3
Linoleic acid ($C_{18:2}$)	80.4	81.7	14.7	16.5	65.3	76.3	79.5	81.7
TUFA	91.2	91.9	91.2	91.7	91.3	91.1	90.1	92.0
TSFA	8.7	8.1	8.7	8.3	8.6	8.8	9.8	7.9
TUFA/TSFA	10.4	11.4	10.4	11.0	10.6	10.3	9.2	11.6

RI, rhizobacteria inoculation; TUFA, total unsaturated fatty acids; TSFA, total saturated fatty acid

Even though oil content of the cultivars did not increase, oil yields increased in the study. Oil yield significantly correlates with both oil contents and seed yield (Bidgoli et al., 2006; Arslan, 2007), and seed yield increase was approximately 20% more in rhizobacteria inoculated seeds. Therefore, seed yield increase contributed oil yield increases observed in the study.

Fatty acid composition of the control and rhizobacteria inoculated cultivars for both years is given in Table 6. Montola 2000 is a high oleic whereas Linas and Dincer 5-18-1 are high linoleic type safflowers, therefore over 90% of the fatty acids were unsaturated fatty acids. Palmitic acid content of Dincer 5-18-1 was lower, whereas palmitic acid content was higher in Linas for rhizobacteria inoculated seeds at both years, and palmitic acid content between control and rhizobacteria inoculated seeds was also different between the years. Stearic acid content of seeds does not appear to be affected by the rhizobacteria application and was around 2% at both years. Oleic acid contents of Dincer 5-18-1 and Linas decreased, but linoleic acid contents of these cultivars increased with rhizobacteria inoculations at both years. Total unsaturated to saturated fatty acids ratios were lower in control seeds for Dincer 5-18-1, but that ratio was higher in control seeds of Linas. Montola 2000 showed different oleic and linoleic acids content between the years. Nosheen et al. (2018b) reported that rhizobacteria inoculations increased oleic acid and decreased linoleic acid contents of the cultivars. In our study, while oleic acid contents in linoleic types decreased, linoleic acid contents increased with rhizobacteria inoculations at both years, and oleic type Montola 2000 showed different responses depending on year but not rhizobacteria inoculation. Total unsaturated fatty acids to saturated fatty acids content ratios improved in Dincer 5-18-1, but decreased in Linas at both years of the research. It was reported that nitrogen fixing rhizobacteria inoculations improved unsaturated/saturated fatty acid ratios in sunflower and safflower (Choudhury and Kennedy, 2004; Nosheen et al., 2018b). However, results of the study showed that unsaturated/saturated fatty acids ratio was dependent on the cultivars used and is influenced by the growth years (Table 6).

To increase safflower cultivation, it is necessary to increase seed yields of the cultivars either through breeding or through cultural practices. Breeding is a long process, but changing cultural practices could be implemented faster. Rhizobacteria applications have been shown to improve nutrient availability, root growth and nutrient acquisition by the plants, which in turn, improve plant growth, yield and quality. In the present study, the effects of nitrogen fixing and phosphate solubilizing *Bacillus* spp. were investigated on agronomic and quality parameters, and rhizobacteria applications increased 1000-seed weight, seed and oil yields under unfertilized rain fed growth conditions. Results of the study showed that rhizobacteria inoculations allowed to obtain comparable yields without the use of chemical fertilizers, and the seed yields could be further increased with a minimal/reduced amount of fertilizers use for economical, sustainable and environmentally friendly agricultural practices for safflower production.

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