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EFFECTS OF BIO- AND CHEMICAL-ORGANIC FERTILIZERS ON YIELD, SOME PHYSIOLOGICAL TRAITS AND FATTY ACIDS COMPOSITION OF CANOLA

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Abstract

Effects of bio- and chemical-organic fertilizers on some physiological traits and fatty acids composition in canola (*Brassica napus* L.) was studied employing a factorial RCBD design in 2016. Experimental factors included four bio- fertilizers levels, such as (i) no bio-fertilizer (B_1), (ii) seed inoculation with *Nitrobacter* (B2), (iii) *Pseudomonas* (B_3), (iv) *Azosprillum* (B_4) and five levels of nitrogen and farmyard manure (FYM) *viz.* (i) 100% nitrogen (F_1), (ii) 25% FYM+75% nitrogen (F_2), (iii) 50% FYM+50% nitrogen (F_3), (iv) 75% FYM +25% nitrogen (F_4) and (v) 100% FYM (F_5). The unsaturated fatty acids (oleic acids, linoneic, linoleni and palminoleic acid) increased at 50% N+50% FMY along with bio-fertilizer compared to the control, while it was found vice versa in case of saturated fatty acids (palmetic and arashidic acid). Application of 100% FYM decreased oil (low erucic acid) and enhances the seed protein. Application of 50% FYM + 50% nitrogen along with *Pseudomonas* treated plants caused an increase of about 111% in seed yield of canola in comparison to that found in B_1F_5.

Introduction

Canola (*Brassica napus* L.) is one of the most important annual oil and protein crops in the world (Sun *et al.* 2017). Canola oil seed species currently hold the third position among oil seed crops and has lowest saturated fatty acids content (Ashraf and Mcneilly 2014). Oil seed production has a fundamental dependence on inorganic N with a current requirement for N fertilization. Improved varieties of canola or hybrids are capable of higher yields when grown under optimum fertility level. Nitrogen plays vital role in its healthy growth and is one of the main precursors of protein which absorbs in the form of mineral, ammonium or nitrate by canola plant. Nitrogen is involved in vital plant functions such as photosynthesis, DNA synthesis, protein formation, respiration and N_2 fixation would directly influence plant growth and development (Caliskan *et al.* 2008). Grant *et al.* (2011) investigated rapeseed response to different levels of nitrogen fertilizer and stated that oil yield increased with low to moderate N rates, but stabilized or fell with high N rates, while chlorophyll content increased with increasing N rates. Excessive nitrogen fertilization on plant not only generates environmental risk, it may also affect the grain quality, decreasing its oil content and reduce plant yield (Caliskan *et al.* 2008). N deficiency is associated with change in fatty acid composition, decreased levels of undesirable long-chain fatty acids and increase in the linoleic and oleic acid contents (Sawan, *et al.* 2006). Physiological efficiency of nitrogen in fall canola can reduce environmental pollution and enhance the economic income. To improve this efficiency and reduce environmental pollution, several integrated fertilizer management strategies have been developed (Sayyad-amin and Ehsanzadeh 2008). Application of bio fertilizers is important to develop fertilization practices that can optimize the N fertilizer rates and decrease

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adverse environmental effects, increase of soil organic matter, improvement of soil properties and increase of crop yield (Adesemoye *et al.* 2009).

Plant growth promoting rhizobacteria, such as *Nitrobacter*, *Azosprilium* and *Pseudomonas* can convert atmospheric N_2 into plant available form of N in the soil (Ahmed *et al.* 2014). Shehata and El-Khawas (2003) reported that oil contents and seed yield in sunflower significantly increased in response to a bio-fertilizer application as compared to the control. Noorieh *et al.* (2013) reported that PGPR species like *Azotobacter* and *Pseudomonas* increased the physiological and biochemical traits of *Brassica napus* L. under normal and stress condition. Heidari and Golpayegani (2012) suggested that PGPR inoculation enhanced photosynthetic pigments and RWC of basil (*Ociumum basilicum* L.).

In the recent years, among the various sources of organic manure, efficacy of manure fertilizer was reported to be manifold. In a broad sense, manure management relates to the appropriate use of animal manure according to each farm's capabilities and goals while enhancing soil quality, crop nutrition, and farm profits. Manure management is defined as a decision-making process aiming to combine profitable agricultural production with minimal nutrient losses from manure, for the present and in the future (Karmakar *et al*. 2007). Chemical fertilizers combined with organic manures result in reduction of soil nitrate contents (Sabagh *et al.* 2015), increase of soil organic matter and increase of crop yield. Application of organic, bio- and chemical fertilizers increased grain yield and agronomic traits of canola (Sabagh *et al*. 2015). Mondal *et al*. (2015) reported that organic and chemical fertilizer along with bio-fertilizer greatly influences quantitative and qualitative characters of mustard. Determination of chemical-organic and bio-fertilizers effects on yield, oil and protein contents of canola is very important to maximize yield and economic profitability of canola production in a particular environment. Moreover, it seems that there is little investigation about the combined effects of bio, chemical-organic fertilizers on these traits of canola. Considering the above facts, the present study was undertaken to elucidate the effects of bio- and chemical-organic fertilizers and seed inoculation with bio-fertilizers on some physiological characteristics and oil components of canola.

Materials and Methods

A factorial experiment based on RCBD with three replications was conducted in 2016**.** Experimental factors included four bio-fertilizers levels, such as (i) no bio-fertilizer (B_1), (ii) seed inoculation with *Nitrobacter iranicum* strain 101 (B_2), (iii) *Pseudomonas putida* strain 186 (B_3), (iv) *Azosprillum lipoferum* strain OF (B_4); and application of nitrogen and FYM (farmyard manure) at five levels, *viz.* (i) 100% nitrogen (F_1), (ii) 25% FYM+75% nitrogen (F_2), (iii) 50% FYM + 50% nitrogen (F_3), (iv) 75% FYM + 25% nitrogen (F_4) and (v) 100% FYM (F_5). Chemical pure nitrogen was prepared from source of urea fertilizer (46% pure nitrogen). *Nitrobacter, Pseudomonas* and *Azosprillum* were isolated from the rhizospheres of canola by Research Institute of Soil and Water, Tehran, Iran. For inoculation, seeds were coated with gum Arabic as an adhesive and rolled into the suspension of bacteria until uniformly coated. The strains and cell densities of microorganisms used as PGPR in this experiment were 1×10^8 colony forming units (CFU).

The experimental area is located at 38°15" N latitude and 48°15" E longitude with an elevation of 1350 m above mean sea level. Climatically, the area is situated in the wet zone with moderate winter and hot summer in north-western Iran. The experiment was carried out on the soil with a texture of silty clay, Haplic Cambisol with pH about 8.2 and electrical conductivity about 2.68 ds/m. In each plot there were 6 rows 3.5 m in length. Seeds were sown on 25 September, 2015.

Fatty acids were extracted based on AOAC (1990) protocol. Nitrogen concentration of seeds was determined by Kjeldal analysis. The protein amount was calculated by multiplying the nitrogen concentration by 6.25. For determination of grain yield, three central rows each of 1 m long were harvested in each plot and converted to grain yield (ton/ha).

Chlorophyll content measured in 0.2 g fresh leaf tissue, which gradually worn with 80% acetone and the solution volume was brought to 20 ml using acetone 80%. Next it was centrifuged for 10 min at 4000 rpm and the absorbance at 645 and 663 nm was recorded by a spectrophotometer. Chlorophyll was obtained based on the following equations (Arnon 1949).

Chlorophyll a = (19.3 × A663 - 0.86 × A645) V.100 W

Chlorophyll b = (19.3 × A645 - 3.6 × A663) V.100 W

The quantum yield was measured by the uppermost fully expanded leaf using a fluorometer (chlorophyll fluorometer; Optic Science-OS-30 USA) (Moludi *et al.* 2014). SPAD index of leaves was determined with a SPAD-502 (Konica Minolta Sensing, Osaka, Japan). The fully developed leaf of plants was randomly selected from five plants of each plot for determination of stomata conductance with leaf prometer (Model SC-J Eijkelkamp, Netherlands).

Results and Discussion

The results indicated that chemical-manure fertilizers and seed inoculation with bio-fertilizers had significant effects on yield, physiological traits and fatty acids composition of canola (*Brassica napus* L.) Analysis of variance showed a significant effect for the bio-fertilizer on protein content (Table 1), chlorophyll a, chlorophyll b, stomata conductance and grain yield (Table 3). Protein content (Table 1), SPAD, Fv. Fm, stomata conductance and grain yield (Table 3) were affected by chemical-organic fertilizers application. Interaction of bio- and chemical-organic fertilizer significantly affected fatty acids composition of canola (Table 1).

Results showed that palmitic acid (C_{16}) was the dominant saturated fatty acid, and oleic acid [18 : 1 (9C)] was the most abundant unsaturated fatty acids, ranging between 63.25 and 71.33 %, followed by linoleic acid [18 : 2 (9C,12C)] and linolenic acid with contents of 9.51–12.55% and 7.33 - 11.04% in different application of bio- and organic-chemical fertilizers (Table 2). The saturated fatty acids (palmetic and arashidic acids) declined in plant treated with bio, chemical-organic fertilizer than the control, while it was *vice versa* in unsaturated fatty acids (oleic acids, linolenic, linoleic and palminoleic acid). Long chain saturated fatty acids are less active than their long-chain unsaturated counterparts (Zheng *et al.* 2005). Application of bio fertilizer as B_3 with the combination of 50% FYM + 50% nitrogen showed the highest oleic acid (Table 2). Significantly higher linoleic acid content (about 31.9% than control) was observed in *Azospirillum* (B_4) and 50% FYM +50% nitrogen (B_4F_3). The lowest per cent of oleic and linoleic acids were recorded in the treatment of 100% N and no bio-fertilizer application (Table 2). Similar results were recorded by Yasari and Patwardhan (2007) who reported maximum oil content in canola by the application of bio-fertilizer and chemical fertilizers. Luís *et al.* (2013) reported that inoculation with bio fertilizers enhanced unsaturated fatty acids content of soybean seeds. Mean comparison between bio and organic-chemical fertilizers showed that the maximum of linolenic acid (11.04) was obvious in the treatment 50% FYM + 50% nitrogen (F_3) in *Pseudomonas* application (B_3F_3), while the minimum value (7.33) were observed in B_1F_2 (Table 2). Means comparison indicated that application of bio- and chemical-organic fertilizers as B_4F_5 increased about 50.61% in linolenic acid content in comparison with B_1F_1 (Table 2). The highest values for palminoleic acid (0.34) was observed in the treatment 50% FYM+ 50% nitrogen while the lowest value (0.15) was observed in the treatment 100% nitrogen with no bio-fertilizer application. It seems that 50% N along with 50% FYM to be the most appropriate and suitable to enhance the canola oil properties.

The findings of the present study are at par with that of Nosheen *et al.* (2013), who reported that *Azospirillum*, in parallel with chemical fertilizer, significantly improved oleic acid, linolenic acid and protein content but reduced erucic acid and improved the oil quality of canola. It was found that, when there was an adequate supply of N in the soil, leaf senescence was slower and the plant was able to supply its seeds with photoassimilate for a longer period (Diacono *et al.* 2013), which might result in higher oil content.

Table 1. Effects of application of bio- and chemical-organic fertilizers on quality indicators of canola.

Treatment	Oleic acid (%)	Linoleic acid (%)	Erucic acid (%)	Arashidic acid (%)	Linolenic acid (%)	Palmitic acid (%)	Palmi-noleic acid (%)	Protein content (%)
Bio fertilizers								
B_1 = No inoculation as control	67.97b	10.80b	2.47a	0.91a	8.71c	4.51a	0.27a	14.99c
B_2 = *Nitrobacter*	27.60b	11.21a	2.35b	0.86b	8.39d	4.46b	0.23c	15.49b
B_3 = *Pseudomonas*	69.58a	10.65ab	2.33b	0.85b	9.96a	4.34c	0.28a	16.32a
B_4 = *Azosprillum*	67.68b	10.90ab	2.30b	0.79c	8.95b	4.34d	0.26ab	16.21a
LSD 5%	0.59	0.37	0.057	0.025	0.18	0.019	0.0083	0.125
Chemical-organic fertilizers								
F_1 = 100 % nitrogen	64.82c	9.54c	2.73a	0.88a	9.17b	4.46a	0.18e	14.93d
F_2 = 25% FYM + 75% nitrogen	68.71b	10.71b	2.33c	0.87a	7.99d	4.45a	0.24d	16.065b
F_3 = 50% FYM + 50% nitrogen	68.38b	12.27a	2.61b	0.84a	0.14b	4.46a	0.30a	16.15a
F_4 = 75% FYM + 25% nitrogen	68.49b	11.02b	2.21d	0.86ab	8.61c	4.46a	0.29b	15.68c
F_5 = 100% cow manure	70.66a	10.89b	1.93e	0.83b	10.10a	4.34b	0.27c	15.95b
LSD 5%	0.66	0.41	0.063	0.028	0.20	0.022	0.0093	0.140
Bio-fertilizers (B)	**	*	**	**	**	**	**	**
Fertilizer (F)	**	**	**	**	**	**	**	**
B × F	**	*	**	**	**	**	**	ns

ns and *,** show no significant and significant differences at 0.05, 0.01 probability level, respectively.

As shown in Table 2 the highest palmitic acid (4.57) and arashidic acid (0.96) in oil were recorded in the treatment 100% N and without bio-fertilizer application. Inoculation with bio-fertilizers as B_2 and application of 100% FYM decreased content of palmitic acid (about 5.68% than control) and arashidic acid (about 21.87% than control). These results are in agreement with the findings of Akbari *et al.* (2011). The highest erucic acid (2.90%) was recorded in the treatments 100% nitrogen (F_1) and no application bio-fertilizer. The minimum oil content (1.84%) was recorded in the treatment of *Azosprillum* and 100% N (Table 2). Lower erucic acid, palmitic acid and arashidic acid in application of organic fertilizer were related to higher protein content of seed, which agrees with the previous reports on sunflower (Shehata and El-khawas 2003). It was reported that optimum nitrogen rates increase cell wall thickness, protein synthesis (Saneoka *et al.* 2004) and cause the reduction of fatty acid. This decrease might have resulted from an increase in allocation of assimilates into amino acid, protein and enzyme production in response to nitrogen application (Sayyad-amin and Ehsanzadeh 2008). Akbari *et al.* (2011) also reported the increase in grain protein content and seed quality of sunflower with the application of bio-fertilizers, nitrogen fertilizer and farmyard manure.

Table 2. Means comparison of chemical-organic and bio fertilizer treatments on fatty acids composition of canola.

Treatment		Oleic acid (%)	Linoleic acid (%)	Erucic acid (%)	Arashidic acid (%)	Linolenic acid (%)	Palmitic acid (%)	Palminoleic acid (%)	Protein content (%)
Bio-fertilizers	Chemical -organic fertilizers								
B_1	F_1	63.25 ± 12.65	9.51 ± 1.90	2.90 ± 0.58	0.96 ± 0.19	9.88 ± 1.98	4.57 ± 0.91	0.15 ± 0.03	14.23 ± 2.84
	F_2	69.50 ± 13.90	10.24 ± 1.05	2.81 ± 0.56	0.93 ± 0.19	7.33 ±1.47	4.50 ± 0.91	0.27 ± 0.05	15.39 ± 3.07
	F_3	66.72 ± 13.34	12.21 ± 2.44	2.89 ± 0.58	0.84 ± 0.17	8.06 ±1.61	4.46 ± 0.89	0.33 ± 0.07	15.28 ± 3.05
	F_4	70.05 ± 14.01	11 ± 2.20	1.93 ± 0.39	0.94 ± 0.19	8.66 ±1.73	4.54 ± 0.91	0.29 ± 0.06	15.03 ± 3.00
	F_5	70.34 ± 14.06	11.06 ± 2.21	2.06 ± 0.41	0.91 ± 0.18	9.64 ±1.93	4.43 ± 0.89	0.31 ± 0.06	15.05 ± 3.01
B_2	F_1	64.98 ± 13.93	9.79 ± 1.12	2.88 ± 0.58	0.90 ± 0.18	7.77 ± 1.55	4.47±0.87	0.18 ± 0.04	15.12 ± 3.02
	F_2	66.86 ± 14.45	11.11 ± 2.22	1.90 ± 0.38	0.88 ± 0.18	8.04 ± 1.61	4.55±0.91	0.22 ± 0.04	14.75 ± 2.95
	F_3	69.42 ± 13.88	12.19 ± 2.44	2.81 ± 0.56	0.85 ± 0.17	7.42 ± 1.48	4.51±0.90	0.25 ± 0.05	15.79 ± 3.15
	F_4	66.56 ± 11.40	11.55 ± 1.93	2.31 ± 0.46	0.85 ± 0.17	8.64 ± 1.73	4.46±0.91	0.29 ± 0.06	15.52 ± 3.10
	F_5	70.21 ± 14.04	11.39 ± 3.67	1.89 ± 0.38	0.75 ± 0.15	10.11 ± 2.02	4.31±0.86	0.21 ± 0.04	16.28 ± 3.25
B_3	F_1	66.93 ± 13.38	9.77 ± 1.95	2.69 ± 0.54	0.85 ± 0.17	8.67 ± 1.73	4.35 ± 0.87	0.18 ± 0.04	15.63 ± 3.12
	F_2	70.34 ± 14.06	11.07 ± 2.21	2.49 ± 0.50	0.81 ± 0.16	8.62 ± 1.72	4.33 ± 0.87	0.24 ± 0.05	17.26 ± 3.45
	F_3	71.33 ± 14.26	12.15 ± 2.43	2.11 ± 0.42	0.90 ± 0.18	11.04 ± 2.21	4.38 ± 0.88	0.34 ± 0.07	17.20 ± 3.44
	F_4	68.34 ± 13.66	10.05 ± 2.01	2.18 ± 0.44	0.91 ± 0.18	9.75 ± 1.95	4.34 ± 0.87	0.32 ± 0.06	15.71 ± 3.14
	F_5	70.99 ± 14.19	10.22 ± 2.04	1.84 ± 0.37	0.80 ± 0.16	11.72 ± 2.34	4.32 ± 0.86	0.33 ± 0.07	15.83 ± 3.16
B_4	F_1	64.13 ± 14.19	9.10 ± 1.82	2.55 ± 0.51	0.87 ± 0.17	10.37 ± 2.07	4.47 ± 0.89	0.23 ± 0.05	14.75 ± 2.95
	F_2	67.14 ± 12.82	10.45 ± 2.09	2.04 ± 0.41	0.82 ± 0.16	7.98 ± 1.60	4.35 ± 0.87	0.23 ± 0.05	16.86 ± 3.37
	F_3	67.05 ± 13.42	12.55 ± 2.51	2.66 ± 0.53	0.77 ± 0.15	10.05 ± 2.01	4.50 ± 0.90	0.30 ± 0.06	16.35 ± 3.27
	F_4	69.01 ± 13.41	11.50 ±2.30	2.45 ± 0.49	0.84 ± 0.17	7.42 ± 1.48	4.56 ± 0.91	0.28 ±0.06	16.48 ± 3.29
	F_5	71.11 ± 14.22	10.92 ± 2.18	1.96 ± 0.39	0.78 ± 0.17	8.96 ± 1.79	4.30 ± 0.86	0.26 ± 0.05	16.65 ± 3.33
$LSD_{0.05}$		1.33	0.83	0.127	0.056	0.41	0.043	0.018	0.281

B_1, B_2, B_3 and B_4 indicate no inoculation as control, *Nitrobacter*, *Pseudomonas* and *Azosprillum,* respectively. F_1, F_2, F_3, F_4 and F_5 indicate 100% nitrogen , 25% FYM + 75% nitrogen , 50% FYM + 50% nitrogen, 75% FYM + 25% nitrogen and 100% FYM respectively. ± indicate standard error.

As shown in Table 3 organic manure in combination with synthetic fertilizers alone or with bio-fertilizers significantly increased SPAD (chlorophyll index) and Fv/Fm ratio (maximum efficiency of PSII photochemistry). Relative chlorophyll content has a positive relation with photosynthetic rate. This positive effect of fertilizers on the photosynthetic pigments may be due to the improvement of chlorophyll formation and photochemical efficiency of leaf. Maximal quantum yield can reflect the photosynthetic activity of the leaves and it is often used to analyze the photosynthesis and related mechanisms in a plant.

Results show that the maximum SPAD (53.20) and Fv/Fm ratio (0.83) were recorded in the treatment of B_3F_3 (Table 4). Minimum SPAD (26.10) and Fv/Fm ratio (0.46) were found in the treatment of 100% N and no bio-fertilizer application.

Table 3. Effects of application of bio- and chemical-organic fertilizers on yield and physiological traits of canola.

Treatment	SPAD	Fv/Fm	Chl. a (mgg FW)	Chl. b (mgg FW)	Stomata conductance (mmol.m^2.s)	Grain yield (ton/ha)
Bio-fertilizers						
B_1 = no inoculation as control	31.83d	0.60d	17.15c	4.73c	13.13c	1.16c
B_2 = *Nitrobacter*	41.95c	0.66c	20.90b	5.54b	19.05a	1.37a
B_3 = *Psedomonas*	49.21a	0.75b	22.22ab	6.34a	17.60b	1.19c
B_4 = *Azosprillum*	46.72b	0.77a	22.83a	6.22a	18.58a	1.26b
LSD 5%	1.21	0.016	1.50	0.17	0.49	0.038
Chemical-organic fertilizers						
F_1 = 100 % nitrogen	39.47c	0.65d	19.95bc	4.83d	16.40c	1.11d
F_2 = 25% FYM + 75% nitrogen	43.73b	0.65d	19.53c	4.86d	14.85c	1.24c
F_3 = 50% FYM + 50% nitrogen	45.67a	0.79a	23.33a	6.86a	20.05a	1.61a
F_4 = 75% FYM + 25% nitrogen	40.72c	0.69c	21.45b	6.25b	18.60b	1.29b
F_5 = 100% FYM	42.55b	0.71b	19.62c	5.73c	15.54d	0.97e
LSD 5%	1.35	0.018	1.68	0.194	0.55	0.043
Biofertilizers (B)	**	**	**	**	**	**
Fertilizer (F)	**	**	**	**	**	**
B × F	**	**	ns	**	**	**
CV (%)	3.87	3.20	9.78	4.12	3.92	4.16

On the other hand, reduction of chlorophyll finally resulted in decrease in the efficiency of photosynthesis. The leaf photosynthesis and chlorophyll content significantly decreased due to unsatisfactory availability of N (Salvagiotti *et al.* 2009, Diacono *et al.* 2013). Sogut (2006) stated that supremacy of organic N along with N can be explained by the fact that FYM is already in the organic reduced form and hence more readily available for plant metabolism. By contrast, plant must spend a lot of energy to take up nitrates when chemical N is applied and reduce them to the level of ammonia. Increasing photosynthetic rate with chemical-organic N fertilization can be attributed to the increasing amount of chlorophyll index, since N is one of the main components of chlorophyll (Salvagiotti *et al.* 2009). The present results showed that plants inoculated with bio fertilizer showed higher chlorophyll content than non-inoculated plants. The highest chlorophyll-b content (7.90 mg/g FW) was obtained with the application of *Pseudomonas* and 50% FYM+ 50% nitrogen. Whereas, the lowest value (3.40 mg/g FW) was observed in application of 100 % nitrogen and non treated plants (Table 4). There was an increase of about 33.11% in content of chlorophyll a in *Azosprillum* inoculation in comparison to the control (Table 3). Previous studies reported that chlorophyll content is higher in bio-fertilizer treated plants (Belimov *et al.* 2009, Khalilzadeh *et al.* 2016). It is defined that *Nitrobacter* and *Azosprillum* inoculation increase stomatal conductance (Table 3), which can lead to increase in photosynthesis and, possibly, an increase in chlorophyll a and chlorophyll b content (Table 3). Khalilzadeh *et al.* (2016) reported that chlorophyll and Fv/Fm increased by the application of bio-fertilizer. Also, it has been suggested that higher stomatal conductance in application of 50% FYM manure + 50% nitrogen and seed treated with biofertilizers lead to higher chlorophyll index and Fv/Fm ratio (Table 4). The increased level of total chlorophyll concentration in plants of treated with the mention component

Table 4. Means comparison of chemical-organic and bio-fertilizer on yield and physiological traits of canola.

Treatment		SPAD	Fv/Fm	Chlorophyll b (mg.g FW)	Stomata conductance (mmol.m^2.s)	Grain yield (ton/ha)
Biofertilizers	Chemical and organic fertilizers					
B1	F_1	26.10 ± 5.22	0.46 ± 0.09	3.40 ± 0.68	10.06 ± 2.05	1.026 ± 0.20
	F_2	34.68 ± 6.93	0.67 ± 0.13	4.26 ± 0.85	13.06 ± 2.65	1.23 ± 0.25
	F_3	38.40 ± 7.68	0.77 ± 0.15	5.79 ± 1.15	14.80 ± 3.00	1.44 ± 0.29
	F_4	27.80 ± 5.56	0.47 ± 0.09	5.12 ± 1.02	15.23 ± 3.05	1.29 ± 0.26
	F_5	32.20 ± 6.44	0.65 ± 0.13	5.12 ± 1.02	12.50 ± 2.50	0.84 ± 0.17
B2	F_1	45.88 ± 9.17	0.62 ± 0.12	4.41 ± 0.88	18.10 ± 3.60	1.45 ± 0.29
	F_2	34.80 ± 6.96	0.66 ± 0.13	5.62 ± 0.12	16.30 ± 3.30	1.27 ± 0.25
	F_3	46.10 ± 9.22	0.75 ± 0.15	6.33 ± 1.26	22.60 ± 4.50	1.52 ± 0.30
	F_4	41.00 ± 8.20	0.74 ± 0.14	5.81 ± 1.16	22.26 ± 4.45	1.43 ± 0.29
	F_5	42.00 ± 8.40	0.52 ± 0.10	5.57 ± 1.11	16.00 ± 3.20	1.22 ± 0.24
B3	F_1	52.37 ± 10.47	0.75 ± 0.15	4.87 ± 0.97	14.80 ± 3.00	0.95 ± 0.19
	F_2	47.00 ± 9.40	0.76 ± 0.15	4.90 ± 0.98	17.16 ± 3.45	1.26 ± 0.25
	F_3	53.20 ± 10.64	0.83 ± 0.17	7.90 ± 1.54	22.60 ± 4.50	1.78 ± 0.36
	F_4	40.30 ± 8.06	0.76 ± 0.15	7.20 ± 1.44	16.16 ± 3.25	1.09 ± 0.22
	F_5	53.20 ± 10.64	0.70 ± 0.14	6.83 ± 1.36	17.26 ± 3.45	0.89 ± 0.18
B4	F_1	42.00 ± 8.40	0.80 ± 0.16	6.65 ± 1.33	19.66 ± 3.95	0.05 ± 0.21
	F_2	43.90 ± 8.78	0.73 ± 0.14	4.69 ± 0.93	15.90 ± 3.20	1.23 ± 0.25
	F_3	45.00 ± 9.00	0.81 ± 0.16	7.45 ± 1.49	20.20 ± 4.00	1.72 ± 0.34
	F_4	53.80 ±10.76	0.78 ± 0.15	6.89 ± 1.37	20.73 ± 4.15	1.38 ± 0.28
	F_5	48.90 ± 9.78	0.72 ± 0.14	5.43 ± 1.08	16.40 ± 3.30	0.96 ± 0.19
$LSD_{0.05}$		2.71	0.037	0.389	10.10	0.086

B_1, B_2, B_3 and B_4 indicate no inoculation as control, *Nitrobacter*, *Psedomonas* and *Azosprillum* respectively. F_1, F_2, F_3, F_4 and F_5 indicate 100 % nitrogen , 25% FYM + 75% nitrogen , 50% FYM + 50% nitrogen, 75% FYM + 25% nitrogen and 100% FYM, respectively. ± indicates standard error.

might be due to the influence on vital plant function such as photosynthesis, DNA synthesis, chlorophyll synthesis by high Rubisco activity, promoting the synthesis of soluble proteins and enzymes (Diacono *et al.* 2013). Thus application of treatment as B_3F_3 induced a 17% increase of stomata conductance compared to B_1F_1 (Table 4). Also, Liu *et al.* (2000) proposed that some growth regulator affects stomata by regulating potassium channels in guard cells.

The present results showed that bio-organic and chemical fertilizer had significant effects on grain yield of canola. The highest yield (1.78 ton/ha) was obtained in *Pseudomonas* (B_3), application of organic-chemical fertilizer as F_3 (Table 4). The lowest yield (0.84 ton/ha) was determined in 100% FYM (F_5) and without application of bio-fertilizer. Application of 50% FYM+50% nitrogen (F_3) along with *Pseudomonas* treated plants (B_3) caused an increase about 111% in yield in comparison to B_1F_5. Based on these results, the stimulatory effect of bio-organic and chemical fertilizer has been attributed to increase in chlorophyll a and chlorophyll b content in the leaves by plants. It has been suggested that improvement of the grain yield under chemical-organic fertilizer might be associated with the enhanced activity of SPAD and Fv/Fm.

Nitrogen is an essential nutrient in creating the plant growth, as well as many energy-rich compounds which regulate photosynthesis and plant production (Ghassemi-golezani *et al.* 2015), thus influencing grain yield. Sabagh *et al.* (2015) reported that organic matter plays role in decreasing susceptibility to erosion, lead to an increase in the availability of nutrient and increasing the activity of macro and trace elements and this was reflected in increasing seed yield. Similar results were also reported by Oscar *et al.* (2014), who stated that organic fertilizer alone or in combination with synthetic fertilizers significantly increased grain against control. Malhi and Gill (2007) investigated the N requirements of rapeseed and found that N fertilization influenced the grain yield positively. It has been reported that bio-fertilizers produces a variety of growth-promoting substances like indole acetic acid, gibberellins and B vitamins (Oscar *et al.* 2014).

In summary, application of bio- and chemical-organic fertilizers plays an important role on physiological and qualitative traits of canola. The application of bio- and chemical- organic fertilizer decreased the saturated fatty acids (palmitic and arashidic acid) and increased unsaturated fatty acids (oleic acids, linoneic, linoleic and palminoleic acid) compared to untreated plants. Based on the results it can be concluded that 50% N+ 50% FMY along with bio-fertilizer appeared to be the most appropriate and suitable for harvesting a good crop of canola.

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