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Effect of different methods of crop rotation and fertilization on canola traits and soil microbial activity

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Abstract

Cropping systems in farmland areas of Iran are characterized by continuous cultivation of crops with consumption of chemical fertilizers leading to serious soil erosion and fertility decline. Information regarding the simultaneous evaluation of crop rotation and fertilization on the canola traits is not available. Therefore, an experiment was conducted during 2007 - 2010. Experimental designs were arranged in a split plot based on randomized complete block design with three replications. Main plots were consisted of three crop rotations and sub plots were consisted of six methods of fertilization. Results showed that the activities of all soil enzymes were generally higher in the organic manure (compost + farmyard manure) treatment. The highest grain nitrogen (34.2 mg/g), sulfur content (4.43 mg/g) and the lowest N/S were obtained in the green-manured rotation. The highest grain N (38.9 mg/g) was obtained in co application of chemical and organic manure treatment. Grain yield in green-manured rotation and co application of chemical and organic manure treatment were higher than other treatments. Canola-wheat-canola rotation had lower oil content and oil yield than those of other rotations. The highest percentage of oil was obtained from farmyard manure and compost application and the highest oil yield belonged to combined application of chemical and organic fertilizers treatment. Application of organic manure significantly increased unsaturated fatty acids.

Keywords: Compost; Farmyard manure; Oil; Organic fertilizer; Soil enzymes.
Abbreviations: FYM- Farmyard manure.

Introduction

Winter oilseed rape (*Brassica napus* L.) is the dominant oilseed crop in northwest region of Iran and is often grown in short term rotations. In Iran, winter oilseed rape is generally cultivated in a crop rotation including chickpea (*Cicer aritenium* L.) or winter wheat (*Triticum aestivum* L.). Introducing a good rotation for canola can lead to increase grain yield and improve sustainability of canola farms. Maintenance and management of soil fertility is the core of development of sustainable food production systems (Doran et al., 1988). Applying green manures is considered as a good management practice in all agricultural production systems because of increasing sustainability cropping system through reducing soil erosion, improving soil physical properties, increasing soil organic matter and fertility levels (Tejada et al., 2008). To be sustainable, organic farming needs to be self-sufficient in nitrogen (N) through the fixation of atmospheric di-nitrogen (N_2) by legumes (Berry et al., 2002), recycling of crop residues (green manures) (Elfstrand et al., 2007) and the application of farmyard manure (FYM), compost and biofertilizer (Rokhzadi and Toashih, 2011). Proper use of N sources is, therefore, required to optimize the economic yield (Mason and Brennan, 1998) and to minimize the potential for environmental pollution (Aufhammer et al., 1994). While a substantial amount of N is provided by conversion of previous crop residues and soil organic matter into soluble soil N, additional mineral N is a prerequisite for high yields. Several investigations have confirmed that canola following cereal crops yielded substantially lower compared to growing after legume (Christen and Sieling, 1995; Sieling et al., 1997). Compared to cereals, canola requires a higher amount of nutrients. Moreover, available nitrogen and sulfur frequently limits seed yield of canola. Fertilization is one of the soil and crop management practices, which exert a great influence on soil and grain quality (Chander et al., 1998). Compost and FYM are organic sources of nutrients, which increase soil organic matter and enhance soil quality. It is well known that organic amendments have a number of benefits for soil biological properties. Enzyme activities have been indicated as soil properties suitable for use in the evaluation of the degree of soil alteration in both natural and agro-ecosystems. Soil microbial properties have a strong correlation with soil health. Some researchers have already suggested the favorable effects of organic manure application and crop rotation on soil enzyme activities (Chander et al., 1998; Kandeler et al., 1999). The study of combining organic manures in crop rotation is a great potential value to organic agriculture in order to avoid applying chemical fertilizers.

The present research is going to introduce a sustainable soil fertility system and to evaluate the combined effects of organic manure such as green manure, compost, and FYM on canola production and microbiological soil quality indicators in crop rotations.

Results and discussion

Soil enzymes activities

The activities of all enzymes varied significantly among different fertilization methods and crop rotations. Only, urease activity was significantly affected by the two-way interaction of fertilizer × rotation. Crop rotation had no significant effect on alkaline phosphatase (Table 2). The activities of all enzymes were generally higher in the N4 treatment than in the unfertilized and chemical fertilizer treatments (Table 3). There were no differences in phosphatase activity between the compost treatment and the FYM treatment. The protease and phosphatase activities in the N3 treatment were significantly lower than in the FYM and compost treatments. As shown in Table 3, alkaline and acid phosphatase generally increased with compost application. Increased phosphatase activity could be responsible for hydrolysis of organically bound phosphate into free ions, which were taken up by plants. Plants can utilize organic P fractions from the soil by phosphatase activity enriched in the soil–root interface (Yosefi et al., 2011. The increase observed in enzymatic activities due to organic fertilizers amendments are in accordance with previous studies. Martens et al. (1992) reported that addition of the organic matter maintained high levels of phosphatase activity in soil during a long-term study. Giusquiani et al. (1994) found that phosphatase activities increased when compost was added at rates up to 90 t/ha and the phosphatase continued to show a linear increase with compost rates up to 270 t/ha in a field experiment. The highest amount of phosphatase activity was observed in R2 rotation (Table 3). The increases in phosphatase activity that we found with the R2 rotation were comparable to those reported from green manuring with Austrian winter pea in winter wheat production systems in eastern Washington (Bolton et al., 1985) and those found with crimson clover green manuring in a corn production system in North Carolina (Kirchner et al., 1993). There are several reports about soil organic matter increases resulting from legume green manuring, even without any complementary addition of FYM and compost as it is frequently prevalent in organic farm management (Drinkwater et al., 1998). The effect of organic amendments on enzyme activities is probably a combined effect of a higher degree of enzymes stabilization to humic substances and an increase in microbial biomass with increased soil carbon concentration (Martens et al., 1992). This is also indicated by the strong correlation of protease, acid phosphatase and urease with microbial soil C concentrations (Nayak et al., 2007). The protease activity was 80.7 µg/g in canola-wheat rotation (R3). This value increased significantly with green manure application in R2 rotation (Table 3). Dehydrogenase is a very useful soil enzyme as it occurs only in live cells, and it can provide an index of endogenous soil microbial activity since its assay involves no addition of a substrate that would preferentially stimulate any particular group of soil organisms. Dehydrogenase should be very useful for the assessment of soil microbial responses to green manuring because they are believed to be linked primarily with microbial activities that are associated with the initial breakdown of organic materials (Bolton et al., 1985). The activity of dehydrogenase was the lowest in canola-wheat rotation (R3) and was increased by the green manure including rotation (Table 3). Soil microbial metabolism was greatly enhanced due to legume green manure application (Elfstrand et al., 2007). Compost application increased dehydrogenase activity (Table 3). Stronger dehydrogenase activity in compost-applied plots may be due to higher organic matter content (Wlodarczyk et al., 2002). Marinari et al. (2000) reported that a higher level of dehydrogenase activity was observed in soil treated with compost and FYM compared to soil treated with mineral fertilizer. These results were similar to our findings that dehydrogenase in rhizosphere soil of N2 treatment was on average three times higher than that of chemical fertilizer (N3) treatment (Table 3). In addition, the higher organic matter levels in the compost treatments may provide a more favorable environment for the accumulation of enzymes in the soil matrix, since soil organic constituents are thought to be important in forming stable complexes with free enzymes. Soil factors including redox potential (Eh) and pH can affect the rate of enzyme mediated reactions by influencing the redox status and ionization respectively, as well as solubility of enzymes, substrates, and cofactors. In addition, some enzymes may be predominated at specific pH levels. Application of compost and FYM caused a faster and higher soil reduction, and at the same time increased the soil pH. Report of Nayak et al. (2007) showed that soil pH was the lowest in the inorganic fertilizers-amended plots and the highest in compost-amended plots. Soil dehydrogenase activity exhibited a strong negative relationship with Eh and a positive relationship with Fe^{2+} content, suggesting aeration status is the major factor determining the activity (Wlodarczyk et al., 2002). The highest urease activity (48.6 µg/g) was obtained under R2N4 treatment (data not shown). In this treatment, co-application of compost and FYM in green-manured crop rotation (R2) assembles good conditions for urease activity. Higher bulk density could account for this difference. Enzyme activities were shown to be linearly related to soil bulk density (Li et al., 2002). Application of nitrogen fertilizers significantly decreased urease activity, while adding organic and green manure increased its activity. The distinctly greater urease activity in the well-fertilized canola-wheat was probably a consequence of the frequent use of urea as N-fertilizer source in this cropping system. It is concluded that using NH_4^+ containing nitrogen fertilizers in the experiments inhibited the microbial induction of urease activity. It seems greater enzyme activities in green manure-treated soil and organic fertilizer application are the key factors for more effective soil nutrient cycling mechanisms that are so critical to soil productivity, and in turn, essential to the sustainability of low-input and organic farming systems.

Grain nitrogen and sulfur

Analysis of variance showed that crop rotation had significant effects on nitrogen (N), sulfur (S), and grain nitrogen to sulfur ratio (N/S) (Table 4). The highest nitrogen and sulfur and the lowest grain N/S were obtained in the R2 rotation (Table 5). Nitrogen to sulfur ratio is considered as a genetic parameter, but it is also affected by nutritional and environmental factors (Zhao et al., 1998). A slightly increase of this ratio for plants is desirable, but high increase in canola can cause symptoms of sulfur deficiency in the plant and reduce yield (Barker and Pilbeam, 2007). As it was observed, grain yield in R2 rotation was higher than other rotations; the mean comparisons revealed that this treatment had the lowest

Table 1. Chemical characteristics of farmyard manure and compost applied to the soil.

Characteristic	pH	N	P	K	Ca	Mg	Zn	Cu	S
			(%)			(ppm)			
Farmyard manure	7.45	0.47	0.49	0.31	745	1100	12	25	659
Compost	7.2	0.7	1.15	0.51	1950	1890	32	295	2106

Table 2. Effects of crop rotation and fertilization methods on soil enzyme activity.

Source of variance	df	Protease	Acid phosphatase	Alkaline phosphatase	Urease	Dehydrogenase
Block	2	3.3 **	7.3 n.s	193.3 n.s	0.2 n.s	12.4 n.s
Rotation	2	12.4 **	43.4 **	231.3 n.s	6.9 **	43.4 **
Error (a)	4	0.45	4.6	108.2	0.34	6.02
Fertilization	5	1258.6 **	412.3 **	23213.3 **	44.6 **	303.4 **
Rotation × Fertilization	10	66.6 n.s	32.3 n.s	4325.4 n.s	101.2 **	12.3 n.s
Error	30	85.6	8.9	1356.6	5.8	19.4

n.s, * and ** are Non- significant, Significant at the 0.05 and 0.01 probability levels, respectively.

N/S. Other studies show that high increase of nitrogen to sulfur ratio in canola resulted in decreasing grain yield (Zhao et al., 1997). Moreover, sulfur plays an important role in the metabolism of fatty acids, and amino acids having sulfur and leaf chlorophyll, hence, sulfur increasing can lead into grain yield and oil quality improvement (Barker and Pilbeam, 2007; Rahman et al., 2011). Therefore, one of the reasons for reducing grain yield in the R3 rotation is attributed to increase of the N/S. Application of green manure before canola planting provides nitrogen and other nutrition elements. Nitrogen fixation by vetch, which was released in the rhizosphere increased N uptake and grain nitrogen content in canola. R1 rotation was higher than canola-wheat rotation (R3) in nitrogen and sulfur contents. This can be justified that R1 rotation crops have absorbed nutrients from different forms and soil depths. This prevents depletion of nutrients and causes them restore in soil. Nevertheless, in rotation with wheat (R3), canola, and wheat root uptook specific forms of nitrogen and other elements in the same depth and as a result, nutrient depletion was caused. The results showed significant effect of basal fertilizer on nitrogen, sulfur and N/S ratio (Table 4). The highest seed N (38.9 mg/g) was obtained in N5 treatment (Table 5). This can be due to application of compost and manure along with chemical fertilizers that caused providing higher nitrogen. Moreover, the use of organic fertilizers increased microbial enzyme activity and soil nitrogen availability for plants (Hatch et al., 2007). Comparing to the compost used, FYM contained a higher percentage of sulfur that increased sulfur uptake and provided higher grain sulfur. Sahni et al. (2008) findings showed that compost application increased the grain sulfur and nitrogen. The lowest N/S ratio was observed in N5 treatment. This treatment was also produced the highest yield.

Leaf chlorophyll

Based on variance analysis (Table 4), leaf chlorophyll was significantly affected by different methods of fertilization and crop rotations. Comparison of means (Table 5) showed that the highest leaf chlorophyll was produced in the R2 rotation. Considering existence of the highest nitrogen in this treatment, achieving high rates of chlorophyll was not unexpected. Comparison of basal fertilizer levels showed that the highest chlorophyll content was obtained from N5 treatment (Table 5). According to the key role of elements such as nitrogen and magnesium in chlorophyll structure, it seems that providing these elements is the main reason for increasing leaf chlorophyll. Positive correlations between leaf nitrogen and chlorophyll content have been reported in several studies (Ding et al., 2005; Damata et al., 2002).

Grain yield

Variance analysis showed that crop rotation and fertilizers sources had significant effects on grain yield (Table 4). Means comparison showed that the R2 rotation caused a significant increase in grain yield compared to other treatments and R3 rotation had the lowest yield. Several factors can increase grain yield in R2 rotation. Uptake of nutrients such as nitrogen and sulfur in the application of green manure is one of the factors increasing the grain yield that was demonstrated in this study. In addition, Yadvinder-Singh et al. (2004) attributed increasing the soil fertility to crop rotation. Furthermore, in other studies, many justifications have been presented for yield increase in crop rotation (Rathke et al., 2005). Pests and diseases control (Larkin, 2008), increasing soil biological activity (Larkin, 2008), and rising water use efficiency (Christen and Sieling, 1995) are the important reasons for increasing grain yield in crop rotation. Application of crop rotation along with increasing soil organic matter increases biodiversity and soil biological community (Kamkar and Mahdavi Damghani, 2009). This, in turn, causes storage resources for future generations. For implementation of crop rotation and achievement to sustainable agriculture, farmer should pass transition period and usually yield increase will start after three to five years. Unfortunately, due to farmers' economic problems, crop rotation application is restricted in Iran and most farmers cannot achieve long-term goals during the transition period and only mono cropping, simpler rotations, or chemical inputs usage meet short time-targets (Kamkar and Mahdavi Damghani, 2009). Application of crop rotation can reduce consumption of chemical fertilizers. Findings of Armstrong et al. (1994) showed that applying legumes as canola pre-plant reduced the amount of nitrogen fertilizers (25 kg per hectare). Considering the serious impacts of chemical inputs use, crop rotation can play an important role in making agroecosystems healthy (Kamkar and Mahdavi Damghani, 2009). Comparison of basal fertilizers revealed that the highest grain yield was obtained from N5 treatment (Table 5). Using chemical fertilizers provided phosphorus, sulfur, nitrogen and adding FYM and compost made providing micronutrient for plants. Erhart and Hartl (2003)

Table 3. Mean comparison of soil enzyme activity affected by fertilization and crop rotations.

Treatment	Protease (μg)	Acid phosphatase (μg)	Alkaline phosphatase (μg)	Urease (μg)	Dehydrogenase (μg)
Crop rotation					
(R1)	90.4 b	173.1 b	2822.9 a	37.3 a	45.7 b
(R2)	105.2 a	190.8 a	2872.8 a	34.8 b	48.7 a
(R3)	80.7 c	89.9 c	2842.1 a	29.3 c	45.2 b
Basal fertilizers					
Farmyard manure (N1)	91.5 c	161.4 b	2887.8 b	45.2 a	61.6 b
Compost (N2)	100.4 bc	163.5 b	2898.4 b	40.5 b	62.5 ab
Chemical fertilizer (N3)	72.1 d	154.4 c	2643.4 c	24.9 c	21.7 d
Farmyard + Compost (N4)	119.7 a	221.1 a	3221.4 a	45.9 a	63.8 a
Farmyard+Compost+Chemical (N5)	102.2 b	159.2 b	2765.1 bc	24.1 c	48.7 c
Control (N6)	67.1 d	48.1 d	2659.6 c	22.4 d	20.8 d

Mean values in each column with the same letter(s) are not significantly different using LSD tests at 5% of probability. (R1: chickpea, sunflower, wheat, canola; R2: green manure, chickpea, green manure, wheat, green manure, canola; R3: canola, wheat, canola).

showed that use of compost increased available phosphorus, potassium, magnesium, and sulfur for plant. The compost used in this experiment contained relatively high sulfur content. Sulfur is the element that canola has positive reaction to its application (Zhao et al., 1997). Moreover, organic fertilizers with improving soil physical properties provided suitable conditions for root development (Ouedraogo et al., 2001).

Grain oil and fatty acids

The results showed that crop rotation and basal fertilizers had significant effects on seed oil content and yield (Table 4). There was no significant difference between R1 and R2 rotations. These two rotations had higher oil content and yield than those of R3 rotation (Table 6). It is stated that in implementation of crop rotation either with green manure or without green manure, seed oil content is increased. Means comparisons of basal fertilizers showed that the highest percentage of oil obtained from N1 and N2 treatments and the highest oil yield were belonged to N5 treatment (Table 6). The application of FYM and compost alongside chemical fertilizers in N5 treatment increased N uptake by plants and caused the lowest percentage of oil obtained from this treatment. There are several reports on decreased canola seed oil with increasing N content (Hocking et al., 2002). High consumption of nitrogen fertilizers can cause decrease in carbohydrate availability for oil synthesis but protein synthesis is increased. The physiological reason for the negative correlation is related to the competition for carbon skeletons during carbohydrate metabolism (Rathke et al., 2005). The synthesis of both fatty acids and amino acids requires carbon compounds from the decomposition of carbohydrates. Since the carbohydrate content of proteins is lower than oils (Lambers and Poorter, 1992), increased N supply intensifies the synthesis of proteins at the expense of fatty acid synthesis and thus, reducing the oil content of the seed. In this study, the high correlation (r=0.78) between grain N and oil contents were observed. Application of FYM and compost individually increased seed oil content; however canola oil yield did not increase significantly compared to chemical fertilizer. Combined application of FYM and compost significantly increased oil yield in comparison to chemical fertilizer treatments. Findings of Hao et al. (2004) showed that long-term application of organic inputs like FYM led to increase in seed oil content. Among fatty acids of canola seed, crop rotation had a significant effect only on stearic acid (18:0). Basal fertilizers also had significant effects on linoleic acid (18:2), oleic acid (18:1), palmitic acid (16:0), stearic acid (18:0), and linolenic acid (18:3). Oleic acid was affected by interaction of rotation × fertilization (Table 4). Means comparison showed that application of FYM and compost significantly increased linoleic acids compared to chemical fertilizers (Table 6). The highest amount of oleic acid (62.08) was observed in N2R2 treatment, but the application of chemical fertilizers reduced percentage of oleic acid. The lowest oleic acid (58.11) was obtained from N3R3 treatment (data not shown). These two fatty acids are considered as unsaturated fatty acids. The more unsaturated fatty acids percentage caused the better quality of canola oil (Ahmad and Abdin, 2000). Rathke et al. (2005) findings also showed that increasing nitrogen availability reduces the percentage of oleic acid in grain oil content.

Material and methods

Site specification

The trial was conducted during 2007 - 2010 at the Agricultural Research Center of Sanandaj (ARCS), Kurdistan province, the northwest region of Iran (35º16′ lat. N; 47º1′ long. E, 1405 m above sea level). Soil test properties at the commencement of the study were: pH (6.4), organic carbon (0.98%), extractable P (10 mg/kg), K (257 mg/kg), Ca (604 mg/kg), Mg (29 mg/kg), Zn (6.1 mg/kg), Mn (7.6 mg/kg), and Cu (0.7 mg/kg). The soil type is sandy loam (100 g/kg clay, 460 g/kg silt, and 440 g/kg sand) with a water holding capacity of 272 g/kg. Depth to water table is 20 m. The soil is susceptible to surface sealing and crust formation.

Experimental design

The experimental design was a split plot based on randomized complete block design with three replications. Main plots were three crop rotations including (R1): chickpea (*Cicer aritenium* L.), sunflower (*Helianthus annuus* L.), winter wheat (*Triticum aestivum* L.), and canola (*Brassica napus* L.); (R2): green manure, chickpea, green manure, winter wheat, green manure, and canola; (R3): canola, winter wheat, and canola. The green manure used was a combination of hairy vetch (*Vicia panunica*) and barley

Table 4. Effects of crop rotations and fertilization methods on canola traits.

Source of variance	df	Grain yield	Chlorophyll	Nitrogen	Sulfur	N/S ratio	Oil percent	Oil yield	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
Block	2	77974 **	1.6 n.s	0.419. n.s	0.003 n.s	0.33 n.s	3.27 n.s	11864 **	0.015 n.s	0.003 n.s	0.19 n.s	0.7 *	0.03 n.s
Rotation	2	682294 **	9.78 *	1716.3 **	7.33 **	44.28 **	10.1 n.s	144535 **	1.7 *	0.01 **	2.13 **	0.04 n.s	0.7 *
Error (a)	4	5867.5	1.57	0.71	0.074	0.42	10.2	3952	0.2	0.005	0.57	0.067	0.02
Fertilization	5	260011 **	324.7 **	941.4 **	48.3 **	55.3 **	273.4 **	470364 **	0.012 n.s	0.009 *	89.6 **	14.06 **	0.001*
Rotation × Fertilization	10	11356 n.s	18.1 n.s	5.9 n.s	0.59 n.s	10.6 *	0.41 n.s	2232 n.s	0.001 n.s	0.0002 n.s	2.32 **	0.029 n.s	0.0006 n.s
Error	30	8335	8.2	2.6	0.44	1.4	7.5	36740	0.008	0.001	0.3	0.083	0.0004

n.s, * and ** are Non- significant, Significant at the 0.05 and 0.01 probability levels, respectively.

Table 5. Mean comparison of canola grain yield and some qualitative traits affected by fertilization methods and crop rotations.

Treatment	Grain yield (kg/ha)	Chlorophyll (Spad reading)	Nitrogen (mg/g)	Sulfur (mg/g)	N/S ratio
Crop rotation					
(R1)	2778.5 b	31.9 b	33.6 b	4.02 b	8.54 a
(R2)	3976.8 a	32.9 a	34.2 a	4.43 a	7.78 b
(R3)	2267.2 c	31.3 b	30.7 c	3.86 c	8.51 a
Basal fertilizers					
Farmyard manure (N1)	2642.7 e	30.5 d	28.5 e	3.18 e	9.13 b
Compost (N2)	2825.1 d	30.7 d	30.5 d	3.48 d	8.75 c
Chemical fertilizer (N3)	3284.2 c	33.5 b	37.6 b	5.15 b	7.39 d
Farmyard + Compost (N4)	3667.3 b	32.4 c	35.5 c	4.75 c	7.46 d
Farmyard+ Compost+ Chemical (N5)	4305.2 a	36.9 a	38.9 a	5.47 a	6.91 e
Control (N6)	1312.1 f	28.2 e	25.8 f	2.59 f	10.01 a

Mean values in each column with the same letter(s) are not significantly different using LSD tests at 5% of probability. (R1: chickpea, sunflower, wheat, canola; R2: green manure, chickpea, green manure, wheat, green manure, canola; R3: canola, wheat, canola).

Table 6. Mean comparison of canola oil and fatty acids affected by fertilization methods and crop rotations.

Treatment	Oil percent	Oil yield (kg/ha)	Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid
			(percentage of fatty acids)				
Crop rotation							
(R1)	43.7 a	1072.8 b	5.03 a	1.72 b	60.20 b	22.44 a	9.15 b
(R2)	43.6 a	1349.7 a	4.96 b	1.61 a	61.93 a	22.43 a	9.19 a
(R3)	43.3 a	704.2 c	5.04 a	1.73 b	59.10 b	22.46 a	9.14 b
Basal fertilizers							
Farmyard manure (N1)	46.5 a	913.1 e	4.99 a	1.69 b	61.43 b	22.70 a	9.15 ab
Compost (N2)	46.8 a	1006.5 d	5.01 a	1.69 b	61.20 b	22.81 a	9.17 a
Chemical fertilizer (N3)	42.5 c	1060.0 c	4.98 a	1.72 a	59.15 d	21.64 c	9.13 b
Farmyard + Compost (N4)	44.1 b	1359.7 b	5.02 a	1.68 b	62.52 a	22.80 a	9.18 a
Farmyard+Compost+Chemical (N5)	41.5 d	1466.8 a	5.03 a	1.69 b	59.98 c	22.31 b	9.18 a
Control (N6)	39.9 e	447.6 f	4.99 a	1.68 b	58.22 e	21.42 d	9.15 ab

Mean values in each column with the same letter(s) are not significantly different using LSD tests at 5% of probability. (R1: chickpea, sunflower, wheat, canola; R2: green manure, chickpea, green manure, wheat, green manure, canola; R3: canola, wheat, canola).

(*Hordeum vulgare*). Sub plots were six strategies of supplying the basal fertilizer requirements of canola including (N1): 15 t FYM/ha; (N2): 10 t compost/ha; (N3): 100 kg triple super phosphate/ha + 150 kg Urea/ha; (N4): 7.5 t FYM/ha + 5 t compost/ha, (N5): 7.5 t FYM/ha + 5 t compost/ha + 50 kg triple super phosphate/ha+75 kg Urea/ha and (N6): Control (without fertilizer).

Preparation and performance

Three soil samples were taken from the upper 15 cm layer of the soil profile of each plot and were analyzed for physical and chemical characteristics for fertilizers recommendation (Carter, 1993). The FYM and compost were also analyzed for chemical and nutrients properties (Table 1). FYM, compost, and chemical fertilizer were added to plots before sowing canola. In the third year, canola seeds were planted on September 10. Main plot size was 10×30 m and spaces between main plots were three meters. Weeds were removed manually in all plots.

Crop measurements

In the third year after canola harvesting, seeds were collected to determine the canola grain yield per unit area. Area harvested was 2 × 10 m for each sub plot. Grain yield of canola was adjusted to 9% moisture content. The nitrogen content of the matured seeds was determined by Microkjeldhal method (Bremner, 1996) and sulfur content was determined by method of Chesnin and Yien (1950). Chlorophyll readings were recorded using a hand-held dual wavelength meter (SPAD 502, Chlorophyll meter, Minolta Camera Co., Ltd., Japan) at the flowering stage. The grain lipid was extracted by petroleum ether using Soxhlet (AOAC, 1960). The composition of fatty acids was determined using gas chromatography (model 8700, Perkin-Elmer, USA) equipped with a flame ionization detector (FID). Individual fatty acid content was calculated based on of the area under the chromatography peak, and then each fatty acid was expressed as a percentage of the total fatty acid content (Ahmad and Abdin, 2000).

Soil measurements

For determining the soil enzyme activity in rhizosphere, Protease (EC 3.4.21-24) activity was determined according to Kandeler (1996). Alkaline (EC 3.1.3.1) and acid phosphatase (EC 3.1.3.2) enzymes were measured using *p*-nitrophenyl phosphate disodium (0.115 M) as substrate according to Mandal et al. (2007). Urease (EC 3.5.1.5) activity was measured using 0.5 M urea as a substrate in 0.1 M phosphate buffer at pH 7.1 (Nannipieri et al., 1974). The NH_4^+-N produced by urease activity was determined using a flow injection analyzer (FIAStar, Tecator, S). To account for the NH_4^+-N fixation by soils, NH_4^+-N solutions with concentrations in the range of those released by urease activity was incubated with these spoils. Dehydrogenase activity was determined by the reduction of triphenyl tetrazolium chloride (TTC) to triphenyl formazan (TPF) as described by Serra-Wittling et al. (1995) with some modifications. Briefly, moist soil (2 g) was treated with 2.5 ml of 1% TTC–Tris buffer (pH 7.6), and then was incubated at 37 °C in darkness for 24 h. All enzyme activities values were calculated based on the oven-dry (105 °C) weight of soil.

Statistical analyses

The plant and soil data collected in this study was subjected to analysis of variance (ANOVA). GLM Procedure was used for the analysis of variance and to test differences between treatments. Means comparison was done through LSD test at 5% of probability using a SAS statistical package (SAS Institute, 2002).

Conclusions

Application of green manure in canola rotation (R2) caused increase in grain yield, nutrient uptake, and unsaturated fatty acids. Furthermore, co-application of FYM and compost along with chemical fertilizer significantly increased grain yield. Application of organic manure could lead to the reduction in chemical fertilizers usage and could improve soil biological structure. Therefore, contamination of soil and ground water would be decreased. Moreover, the R2 rotation has biological and environmental efficiencies. In R2N5 treatment using in farm inputs such as green manure, compost and FYM, a low input system can be carried out, and it can help achieving sustainability of farms.

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