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The effect of biofertilizers, nitrogen fertilizer and farmyard manure on grain yield and seed quality of sunflower (*Helianthus annus* L.)

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Experiment was conducted during 2007 growing seasons at the Field of College of Agriculture, Tarbiat Modares University, Tehran Province, Iran to study the effect of biofertilizers (*Azosporillum* and *Azotobacter*), nitrogen fertilizer (N) and farmyard manure (FYM) in various rates on the grain and biological yield, seed protein content as well as seed oil content and fatty acid composition in sunflower (*Helianthus annus L. cv. Alestar*). The results showed that both grain and biological yield produced a better result during the combination of nitrogen fertilizer and farmyard manure than using either method alone. Maximum grain and biological yields of 2823.3 kg $ha^{-1}$and 9917.9 kg ha^{-1} were obtained with the F3 treatment, respectively. The results revealed that biofertilizer improved plant productivity and quality in sunflower seed. The application of biofertilizer (I1) decreased the saturated fatty acids (palmitic and stearic) and increased unsaturated fatty acids (linoleic acid and oleic acid) and oil content, compared with untreated plants I0. The highest linolenic acid (53.28%) and oleic acid (40.65%) were observed in F3 and F1 treatments respectively.

Key words: sunflower, fatty acid, biofertilizer, farmyard manure, chemical fertilizer and integrated system

Introduction

Organic agricultural practices aim to enhance biodiversity, biological cycles and soil biological activity so as to achieve optimal natural systems that are socially, ecologically and economically sustainable (Samman *et al*. 2008). Manure has always been considered as a valuable input to the soil for crop production. 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 adecision-making process aiming to combine profitable agricultural production with minimal nutrient losses from manure, for the present and in the

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future (Karmakar *et al*., 2007). Excessive nitrogen fertilization of sunflower not only generates that environmental risk, it may also affect the grain quality, decreasing its oil content and reduce yield through an increase of plant lodging (Scheiner *et al*., 2002). Sunflower (*Helianthus annuus*), belonging to the family Compositae, is a major oilseed, used for the production of edible oil, with an annual production of 25.1 million tons (FAO 1996).

Sunflower oil is a source of fatty molecules that can be used as reagents for chemical modifications. Sunflower oil also has excellent nutritional properties. It is practically free of significant toxic compounds and has a relatively high concentration of linoleic acid. This polyunsaturated fatty acid is an essential fatty acid (not synthesized by humans), and is the precursor of gamma-linolenic and arachidonic acids (Seiler 2007).The composition of fatty acids is a main determinant of the oil quality in sunflower. Soil microbes play an important role in many critical ecosystem processes, including nutrient cycling and homeostasis, decomposition of organic matter, as well as promoting planthealth and growth as bio-fertilization (Han *et al*., 2007). Certain strains are referred to as plant growth-promoting rhizobacteria (PGPR), which can be used as inoculant biofertilizers (Kennedy *et al*., 2004). These bacteria include species of Azotobacter and Azospirillum, both of which provide direct and indirect effects on plant growth and pest resistance (Persello-Cartieaux *et al*., 2003; Kennedy *et al*., 2004; Nelson, 2004). In recent years, biofertilizers have emerged as a promising component of integrating nutrient supply system in agriculture. Our whole system of agriculture depends in many important ways, on microbial activities and there appears to be a tremendous potential for making use of microorganisms in increasing crop production. Microbiological fertilizers are an important part of enviroment friendly sustainable agricultural practices (Bloemberg *et al*., 2000). The selection of manure management and treatment options increasingly depends on environmental regulations for preventing pollution of land, water and air (Karmakar *et al*., 2007). In this field, many experiments were conducted to study the effect of biofertilizers alone or in combination with other chemical fertilizers (Ram Rao *et al*., 2007; Seema *et al*., 2000). Sunflower hybrid gave a higer yield from a combination of organic manures with chemical fertilizer (Dayal and Agarwal, 1998).

The aim of the present study was to test the effects of application biofertilizers, nitrogen fertilizer and farmyard manure in various rates on the grain and biological yield, seed protein content as well as seed oil content, protein content and fatty acid composition in sunflower (*Helianthus annuus*).

Materials and methods

Location and plant materials

These study was conducted at the Experimental field area of Agronomy Department, Tarbiat Modares University, Faculty of Agriculture, Tehran, Iran (35° 44′N, 51° 10′E , altitude 1352 m), on Sunflower (*Helianthus annuus)*, in the 2007, on a sandy loam soil. Soil samples were taken at 30 cm intervals depth one week before sowing. Mechanical soil analysis and chemical characteristics have been shown in Table 1 and the physical and chemical properties of farmyard manure are presented in Table 2 to provide information about the availability of nutrients in the soil.

The experiment was planted after tilling practice in the region (chisel plow and disking). Row spacing was 50 cm, and plant density was 10 plants per m2. The experimental plot area was 21 m^2 and each plot contained 6 ridges. Two ridges without planting were left between each plot to avoid shading effect. Three seeds per hill were planted. One plant per hill was maintained at 2-4 leaf stage of the crop. The crop exhibited no sign of insect/pest attack and disease incidence; therefore no protection measures were adopted. Crop was planted manually in last week of May and harvested in 20 October 2007. The grain and biological yields were recorded on plot basis and then converted to kg ha^{-1}.

Fertilizer and microbial inocula

All the farmyard manure (FYM) were applied in plots at sowing time for treatments in based of 32 ton/hectare. Nitrogen (as urea) was applied in based of 260 Kg he^{-1} in plots as per treatments in two splits; ½ N at first irrigation and ½ N at flowering stage. Urea was used as sources of inorganic (N) fertilzers. Sunflower seeds were inoculated with biofertilizer and kept for half an hour in shade. The biofertilizers were *Azospirillum* and *Azotobacter*. The both of *Azospirillum* and *Azotobacter* consist of 10^8 DUF g^{-1} inoculat. The inocula were sealed in sterile plastic bags and stored at 4 ℃ for further use, no longer than 3 months. The inocula of the two rhizobacteria species, *Azotobacter* sp. *Azospirillum* sp, were purchased from Mehr Asia Biotechnology Company, Tehran, Iran.

Table 1. Mechanical and chemical analysis of studied soil.

Organic matter (%)	Texture	Clay (%)	Silt (%)	Sand (%)	pH
1.06	Sandy loam	11	20	69	7.6
Total N (%)	Available P p.p.m	Available K p.p.m	Available Fe (mg/kg)	Available Zn (mg/kg)	Available Cu (mg/kg)
0.7	1	7.6	>350	>25	0.07

Table 2. Chemical analysis of fertilizer sample.

Mn (mg/kg)	Fe (mg/kg)	Zn (mg/kg)	Cu (mg/kg)	pH	Organic (carbon)	Total K (%)	Total P (%)	Total N (%)
267/6	7435	109/3	25/5	9	28/85	2/55	0/56	1/25

Pot experiment

This experimental design was a randomized complete block (RCB) design arrangement in a split plot with three replications. Five levels of fertilizers including; F1: (100% farm yard manure); F2: (75% farm yard manure and 25% N); F3: (50% farm yard manure and 50% N); F4: (25% farm yard manure and 75% N); F5: (100% N) were located in main plot units and two levels of biofertilizer as follows:- no biofertilizer (control) and biofertilizer (Inoculation with *Azospirillum* and *Azotobacter*) were randomized to the sub plot units.

Extraction of seed oils

Three individual 10 g samples of crushed dry seeds of each sunflower treatment were refluxed with 300 mL of petroleum benzene in weighed flasks using a Soxhlet apparatus according to the AACC (1987) method. The oils were recovered by distilling the solvent in a rotary evaporator at 45°C, then dried to constant weight in a vacuum oven at 90°C, for 1 h and weighed.

Fatty acid analysis

Fatty acid methyl esters (FAMEs) were prepared, following the procedure described by AOAC (1990). Three aliquots of 0.2 g lipid extract for each treatment were esterified with 10mL methanolic NaOH solution by refluxing for 10 min at 85°C. After addition of internal standard (0.1mL of 2 g/L, C17:0) and 4.4mL of BF3-etherate, the samples were boiled for 2 min.The FAMEs were extracted from a salt saturated mixture with hexane (3.0 mL). For drying FAMEs, anhydride Na_2SO_4 was added and centrifuged (7min, 2500 rpm, Kubota, 6900 Japan). The upper part was poured in specific cell. The esters were separated by GC (Unicam Pu 4550, UK) fitted with a capillary column (FFAP, 25 m, 0.25 mm i.d., 0.22 μm film thickness). Helium was used as carrier gas at inlet pressure of 1.2 kg/cm^2. The temperatures of injection port and detector (FID) were maintained at 200 and 240 1C and the temperature programming for the column was applied as follows, 170°C (4 min), then to 180°C at 31C/min, then to 190°C at 11C/min (25 min). The peaks were identified based on their retention times using authentic standard fatty acids methyl esters and all samples were run in duplicate.

Data analysis

Data were analyzed with analysis of variance (ANOVA) procedures using the SAS software package (SAS Institute, 1998). Differences among the treatments were evaluated with Duncan Multiple Range Test. The $P<0.05$ level was accepted as statistically significant.

Results and discussion

Sunflower grain and biological yield

The different combinations of FYM and N –fertilization treatments had significant effect on grain, biological yield and on the quality parameters such as grain protein content, oil content and percentages of fatty acid in the sunflower seed oil. The highest grain yield (2823.3 kg ha^{-1}) was obtained in the integrated treatment of 50% N + %50 FYM which was statistically significant difference to other integrated treatments of 75% N +25% FYM and 25% N + 75% FYM both giving grain yields of 2521.7 kg ha^{-1} and 2357.5 kg ha^{-1}, respectively. Maximum biological yield (9917.9 kg ha^{-1}) was also obtained in the same treatment. The minimum grain yield (1656.5 kg ha^{-1}) and biological yield (7020.7 kg ha^{-1}) were recorded in the treatment where 100% FYM was applied. N fertilizer (100% N kg ha^{-1}) produced relatively more grain yield than 100% FYM (Table 4). Application of N fertilizers along with FYM increased the grain yield significantly over the treatments. This could be possible increased because of more availability of nutrients and their uptake. Similar findings were also reported by Tiwari and Parihar (1992), Ramesh *et al*. (1999), Gorttappeh *et al*. (2000), Saeed *et al*. (2002), who stated that organic manure alone or in combination with synthetic fertilizers significantly increased grain and biological yield against control.

The application with biofertilizers containing beneficial microbes (*Azotobacter* & *Azospirillum*) showed a promoting effect on the grain and biological yield. Comparing with the uninoculated treatments, the treatment of biofertilizer increased nearly by 9% grain yield and 8% biological yield. Stimulation of different crops by rhizobacterial inoculation has also been demonstrated by other studies both in laboratory and field trials. For example, it was reported that wheat yield increased up to 30% with Azotobacter inoculation and up to 43% with *Bacillus* inoculants (Kloepper *et al*., 1991); It has been reported that biofertilizers not only provides nitrogen, but also produces a variety of growth-promoting substances, among them indole acetic acid, gibberellins and B vitamins (Wu *et al*., 2005).

Seed protein content and yield

The integrated fertilizer significantly increased the seed protein content and protein yield per ha (Table 4). The maximum protein content (20.2%) and protein yield per ha (572.8 kg protein ha^{-1})were recorded in the treatment of 50% FYM + 50% N. Minimum protein content (17.7 %) and protein yield per ha (303.9 kg protein ha^{-1}) were found in the treatment of 100% FYM. These results are similar to those of Ghani *et al*. (2000), Nanjundappa *et al*. (2001), Khaliq (2004) who reported increasing in grain protein content with nitrogen for organic and inorganic sources.

Sugiyama *et al*. (1984) stated that the soluble proteins are increased with better N supply and favorable growth condition. Greef (1994) reported that high values of the reduced N fraction (protein fraction) were found in photosynthetic active leaf tissue. This is true especially under conditions of favorable nitrate supply. These results suggest that the high N-rate increases the amino acids synthesis in the leaves, and this stimulates the accumulation of protein in the seed rather than oil content.

Application of biofertilizer significantly increased the seed protein content (19.5) and protein yield per ha (475.14) over the untreated control (Table 5). These results are in accordance with those obtained by Tiwana *et al*. (1992), Chela *et al*. (1993), Sharma and Namdeo (1999). Seed protein content was increased in response to biofertilizer application to soybean (Sharma and Namdeo, 1999). Bacterial culture (as a biofertilizer) alone or in combination with nitrogen fertilizer increased crude protein (by Tiwana *et al*., 1992; Chela *et al*., 1993).

Seed oil content and yield

In this investigation, it could be concluded that oil content and oil yield per ha sighificantly increased in response to combinations of organic and inorganic fertilization application as being compared to the organic alone or inorganic fertilization. The highest oil content (49.4%) and oil yield per ha (1275.9) were recorded in the treatments 100% FYM and 50% FYM + 50% N respectively. The minimum oil content (45.3%) in the treatment of 100%N and oil yield per ha (819.3) were recorded in the treatment of 50% FYM + 50% N (Table 4). Lower oil content was related to higher protein content of seed, which agrees with previous reports for sunflower (Scheiner *et al*. 2002).

Applications of biofertilizer resulted in a significant increase in seed oil content (47.7) over that of the control (Table 5). The seed oil yield was also significantly increased (1141.5 kg oil ha^{-1}) compared with the untreated control. These results are also in agreement with those obtained by kumar (1994), who found that, using of *Azotobacter chroococcum* as biofertilizer could enhance the oil yield, as compared to the control.

Table 4. Mean comparison for quality and quantity traits of sunflower under different nutrient systems.

Treatments	Yield (kg ha-1)	Biological yield (kg ha-1)	Protein yield (kg ha-1)	Protein (%)	Oil yield (kg ha-1)	Oil (%)
F1	1659.5 d	7020.7 c	303.9 d	17.7 c	819.3 c	49.4 a
F2	2357.5 bc	8732.2 ab	439.4 bc	18.4 bc	1119.0 ab	47.7 b
F3	2823.3 a	9917.9 a	572.8 a	20.2 a	1275.9a	45.3d
F4	2521.7 ab	9107.8 ab	492.6 b	19.5 a	1171.9 a	46.4 cd
F5	2116.0 c	8203.5 bc	407.2 c	19.2 ab	997.3 b	47.1 bc

Means followed by same letters in each column are not significantly different at the 5 %,(Duncan).

Table 5. Mean comparison for quality and quantity traits of sunflower under biofertilizer.

Treatments	Yield (kg ha^{-1})	Biological yield (kg ha^{-1})	Protein yield (kg ha^{-1})	Protein (%)	Oil yield (kg ha^{-1})	Oil (%)
Io	2189.8 b	8252.1 b	411.3 b	18.4 b	1011.9 b	46.6 b
I1	2401.3 a	8940.7 a	475.14 a	19.5 a	1141.5 a	47.7 a

Means followed by same letters in each column are not significantly different at the 5 %,(Duncan).

Oil fatty acids composition

Fats are classified into saturated and unsaturated fats. Saturated fats tend to increase blood cholesterol levels, while unsaturated ones show the reverse direction; they are mostly from plant sources. The most common saturated fatty acids found in plant lipids that contain 16 or 18 carbon atoms. Low content of saturated fatty acids is desirable for edible uses. Usually only palmitic acid (C16) and stearic acid (C18) are present in significant amount, but the saturated fatty acids collectively account for only 20% of the total fatty acid content of most plants, while those with one or more double bonds (unsaturated fatty acids) account for the remaining 80%. In many fatty seeds, oleic [18:1(9C)] and linoleic [18:2 (9C,12C)] acids frequently account for more than 70% of the fatty acid content (›90% in sunflower) (Anderson and Beardall, 1999).

Results showed that palmitic acid was the dominant saturated fatty acid and Linoleic acid was the most abundant of the unsaturated fatty acids. Maximum mean values for oleic acid (40.65) were observed in the treatment 100% FYM, while the minimum values (37.28) were observed in the treatment 50% FYM + 50% N (Table 6). The minimum mean values for linoleic acid (48.06) was observed in the treatment 100% FYM, while the highest values (53.28) was observed in the treatment 50% FYM + 50% N. There is a strong negative relationship between linoleic and oleic acid concentrations, if one increases the other decreases (Seiler 1986, 2007). Thus, the high linoleic concentration of in the treatment 50% FYM + 50% N is accompanied by a corresponding low concentration of oleic acid. The highest palmitic acid (6.2) and stearic acid (4.5) in oil were recorded in the treatment 100% N. The minimum palmitic acid (5.7) and stearic acid (3.2) in oil were recorded in the treatment 100% FYM. The environmental relations between saturated palmitic and stearic fatty acids are less clear than those for linoleic and oleic fatty acids. Kheir *et al*. (1991) found that the higher N-rate increased the percentage of unsaturated fatty acids and decreased the saturated fatty acids of flax oil.

In the present study, no differences in linolenic acid and arachidic acid levels were observed between different combinations of organic (FYM and biofertilizer) and N fertilizers (Table6). The applicationof biofertilizer resulted in a significant decrease in the saturated fatty acids (palmitic, and stearic) while it resulted in a significant increase in the linoleic acid and oleic acid unsaturated fatty acids, compared with untreated plant (Table 7). These results are in line with those of Steer and Seilor (1990) also reported that N supply rates affected on fatty acid composition in sunflower oil. The relation between oil percentage and percentage of oleic acid was negative. Such results may be due to the adverse effect of nitrogen on oil content, is offset by an increase in protein content.

On the basis of results, it can be concluded that the crop was fertilized at 50% N along with 50% farm yard manure and biofertilizer appeared to be most appropriate and suitable for harvesting a good crop of sunflower. It is further noted that the nitrogen fertilizers should not be applied alone, rather in combination with FYM or biofertilizer.

Table 6. Mean comparison for fatty acids of sunflower under different nutrient systems.

Treatments	Palmitic acid (c16:0)	stearic acid (c18:0)	Oleic acid (c18:1)	Linoleic acid (c18:2)	Linolinic acid (c18:3)	Arachidic acid (c20:0)
F1	5.7 b	3.20 d	40.65 a	48.06 d	o.31a	0.93a
F2	5.8 b	3.51 cd	39.58 ab	48.73 cd	0.32a	0.90a
F3	5.8 b	3.65 bc	37.28 c	53.28 a	0.30a	0.84a
F4	6.0 ab	3.93 b	37.63 c	50.60 b	0.31a	0.89a
F5	6.2 a	4.5 a	38.45 bc	50.06 bc	0.32a	0.90a

Means followed by same letters in each column are not significantly different at the 5 %,(Duncan).

Table 7. Mean comparison for fatty acids of sunflower under biofertilizer.

Treatments	**palmitic acidc (16:0)**	**stearic acid (c18:0)**	**Oleic acid (c18:1)**	**Linoleic acid (c18:2)**	**Linolinic acid (c18:3)**	**Arachidic acid (c20:0)**
I0	6.31 a	3.96 a	38.16 b	49.10 b	0.31 a	0.9oa
I1	5.60 b	3.55 b	39.28 a	51.19 a	0. 31a	0.89a

Means followed by same letters in each column are not significantly different at the 5%,(Duncan).

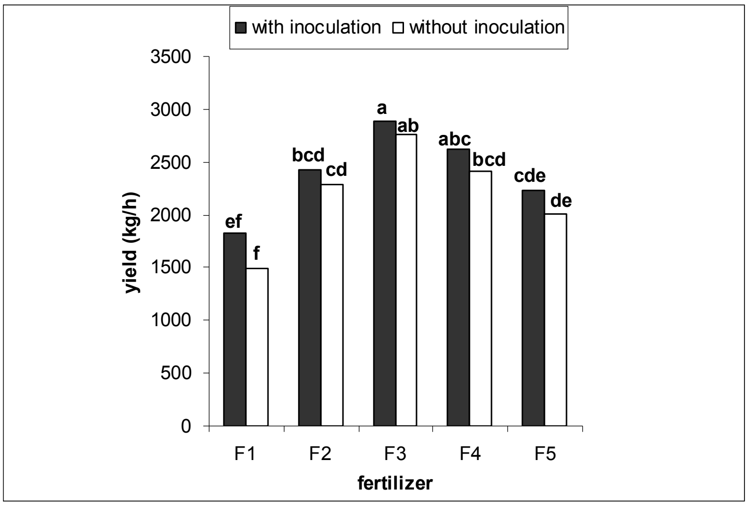

Fig. 1. Interaction effects of inoculum and fertilizer on seed yield of sunflower.

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Table 3. Analysis of variance for different traits of sunflower under different nutrient systems and biofertilizer.

Source of variation	yield	biological yield	Protein yield	Oil yield	palmitc acidc 16:0	stearic acid c18:0	Oleic acid c18:1	Linoleic acid c18:2	Linolinic acid (c18:3)	Arachidic acid (c20:0)
Replication®	56973.7	861295.1	5444.4	22635.8	0.046	0.03	5.92	0.15	0.0006	0.0082
Fertilizer(F)	1155479.3**	6995306.9*	59921.97**	184641.3**	0.27*	1.44**	11.68**	24.56**	0.0005ns	0.0059 ns
Error a	55588.7	1344945.4	2154.4	15603.0	0.05	0.06	1.56	1.07	0.0005	0.0058
Inoculum(I)	335389.1**	3555861.5**	30549.2**	126044.3**	3.78**	1.30**	9.40*	32.86**	0.000003 ns	0.0010 ns
F*I	11266.3 ns	170657.4ns	194.9 ns	3648.3 ns	0.09ns	0.24ns	0.15ns	2.35*	0.0018 ns	0.0195 *
Experimental Error	10001.2	108825.3	303.85	3199.4	0.03	0.07	1.02	0.45	0.0008	0.0045
C.V.	4.3	3.8	3.9	5.2	3.34	7.17	2.61	1.35	8.9	7.54

- and **: Significant at %5 , %1 level of probability, respectively
- ns: Non Significant