

## Integrated Fertilization Systems Enhance Quality and Yield of Sunflower (*Helianthus annuus* L.)

S. Shoghi-Kalkhoran<sup>1</sup>, A. Ghalavand<sup>1\*</sup>, S. A. M. Modarres-Sanavy<sup>1</sup>, A. Mokhtassi-Bidgoli<sup>1</sup>, and P. Akbari<sup>1</sup>

### ABSTRACT

Effects of various fertilizers such as farmyard manure (FM), urea, and plant growth promoting rhizobacteria (PGPR, including the genera *Azotobacter* and *Azospirillum*) were checked on yield, fatty acids, protein, and oil contents of sunflower. A two-year field experiment was conducted in a semi-arid region in Iran in 2007 and 2008. The experiment was a split plot in a randomized complete block design with three replicates, with five fertilizer treatments as the main plots and two PGPR treatments as sub plots. Results demonstrated that the integrated fertilizers significantly increased the leaf area index, plant height, grain production, biomass, oil yield, and protein content in comparison with organic or chemical fertilizers. The maximum and minimum oil contents were obtained by applying 100% farmyard manure (F1) and 50% FM + 50% chemical (F3), respectively; however, opposite results were observed for protein content. When higher FM rates were used with PGPR, the saturated fatty acids (palmitic and stearic acids) declined significantly, while unsaturated ones (linoleic and oleic acids) were increased. The highest contents of linoleic acid (52.6%) and oleic acid (39.8%) were observed in F<sub>3</sub> and F<sub>1</sub> treatments, respectively. Bacterial inoculation also increased the leaf area index, plant height, biomass, grain and oil yields, oil and protein contents up to 12, 3.7, 7.8, 10, 6.5, 5.6 and 5%, respectively.

**Keywords:** Oil crop, Nitrogen, Manure, PGPR, Rhizobacteria.

### INTRODUCTION

Sunflower (*H. annuus* L.) is one of the most common oil crops worldwide (Valtcho *et al.*, 2009). It is also considered as one of the major oil producing crops because of its moderate production requirements, high-quality oil, protein contents and consumable parts of this plant (Škorić, 1992). High percentage poly-unsaturated fatty acids in sunflower oil such as linoleic acid in classical sunflower, which can reach up to 90% of the total unsaturated fatty acids, makes it high-quality edible oil commercially (Schuster, 1993).

Generally, extensive efforts are necessary to improve soil hydro-physical properties as well as its productivity through application of

fertilizers (Helmy and Ramadan, 2009). Fertilizers can improve soil structures and fertility by motivating biological activity and enhancing phosphorus solubility in soil (Hailu *et al.*, 2008). Fertilizer is one of the major factors that could increase sunflower production per unit area. Although excessive nitrogen fertilization can generate environmental hazard, it may also affect sunflower grain quality and decrease its oil content (Merrién and Milan, 1992). Significant reduction of sunflower yield through an increase in biomass is another effect of nitrogen fertilizer overuse (Hussein *et al.*, 1980). In such a situation, organic fertilizers play a major role (Chandrasekar *et al.*, 2005). Organic manures contain valuable nitrogen

<sup>1</sup> Department of Agronomy, Faculty of Agriculture, Tarbiat Modares University, P. O. Box: 14115-336, Tehran, Islamic Republic of Iran.

\* Corresponding author; e-mail: ghalavaa@modares.ac.ir



sources, phosphate, and potash that can be used in the farm for several years. Ming-gang *et al.* (2008) have shown that long-term applications of chemical or organic fertilizers prevent the development of soil structure. Chemical fertilizers combined with organic manures result in reduction of soil nitrate contents (Yang *et al.*, 2005), increase of the cation-exchange capacity and soil organic matter, improvement of soil properties, and increase of crop yield (Mohammad, 1999). Therefore, the integrated fertilizers are a useful means to increase crops yields and enhance the soil fertility (Mahapatra and Sharma, 1989).

Soil microorganisms are likely to have been critical in the in the biogeochemical cycles of both inorganic and organic nutrients in soil and in the maintenance of soil health and quality (Jeffries *et al.*, 2003). Beneficial free-living soil bacteria isolated from the rhizosphere, which have been shown to improve plant health or increase yield, are usually known as plant growth promoting rhizobacteria (PGPR) (Suslow and Schroth, 1982).

The aim of this study was to find the most effective formulation of organic and chemical fertilizers, to maximize the yield and quality of sunflower productions in a semi-arid region.

## MATERIALS AND METHODS

### Plant Materials and Field Experiments

The field trials were performed in 2007

and 2008 at the Faculty of Agriculture Research Station, Tarbiat Modares University, Tehran, Iran (N 35°44", E 51°10"). The field is located in a semi-arid area (according to the Köppen climate classification) with warm and dry summer. Figure 1 shows the daily temperature and precipitation during the two growing seasons.

Soil was sampled 20 days before the beginning of the experiment in the first and second year. Composite soil sampling was performed from top 30 cm depth, and the physical and chemical characteristics were determined in laboratory. The total nitrogen, available phosphorus, and available potassium were measured based on the Kjeldahl method, Olsen method, and ammonium acetate extraction protocol (Tandon, 1995), respectively (Table 1). The total nitrogen rates estimated from each pre-plant fertilization treatment of the second year are shown in Table 2.

Plants received five fertilizer treatments: F<sub>1</sub> (100% farmyard manure (FM) 48 t/ha<sup>-1</sup>), F<sub>2</sub> (75% FM+25% chemical; urea 240 kg/ha<sup>-1</sup>), F<sub>3</sub> (50% FM+50% chemical), F<sub>4</sub> (25% FM+75% chemical), F<sub>5</sub> (100% chemical). Plants were also inoculated with PGPR (I<sub>1</sub>) while non-inoculated plants (I<sub>0</sub>) were used as control treatments. The soil plant available nitrogen required for sunflower from planting to maturity was estimated at 150 kg ha<sup>-1</sup> based on nitrogen content in 0–30 cm depth of the soil, farmyard manure, and

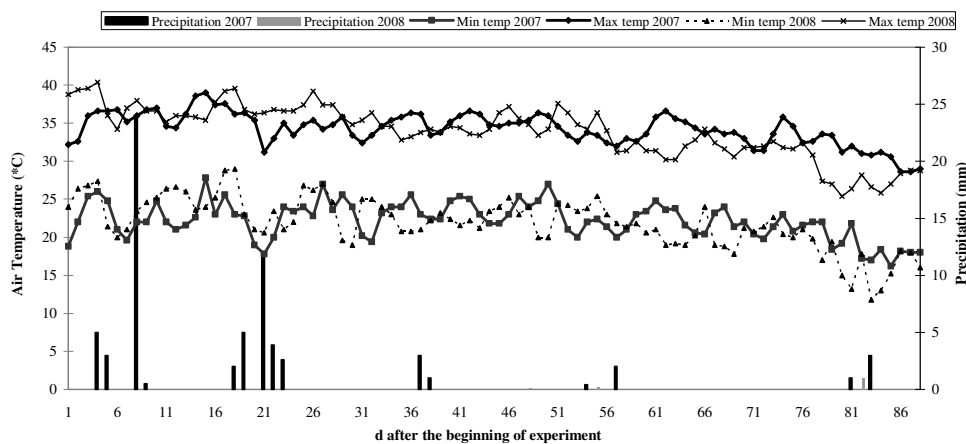


Figure 1. Daily temperature and precipitation during the growing season in 2007 and 2008.

**Table 1.** Chemical and physical characteristics of the soil at the beginning of the experiment (2007).

Property	Results
Sand (%)	69
Silt (%)	20
Clay (%)	11
pH	7.7
Total neutralized materials	5.5
Cation exchange capacity (meq 100 g <sup>-1</sup> )	6.4
Organic matter (%)	1.06
Total nitrogen (%)	0.07
Available potassium (mg kg <sup>-1</sup> )	>350
Available phosphorus (mg kg <sup>-1</sup> )	>25

urea.

In early seed germination, the total farmyard manure and half of the total urea were incorporated into the soil and the remaining urea was top-dressed at the flowering stage.

Seeds were inoculated using nitroxin bio-fertilizer (PGPR), which is a mixture of the most effective nitrogen fixing bacterial species including *Azotobacter* and *Azospirillum*. This liquid bio-fertilizer formulation containing 10<sup>8</sup> cells/ml (CFU) was purchased from the Mehr Asia Biotechnology, Iran. Seeds were treated based on the manufacturer's protocol 1 L 30 kg<sup>-1</sup> seeds.

The sunflower (*cv.* Alestar) seeds were sown at a depth of 5 cm and a density of 5 seeds m<sup>-1</sup> row on 28 June 2007 and 12 July 2008. Plots were prepared 7 m long including 6 rows spaced at 0.5 m. Run off was controlled by making 2m-wide channels between all the main plots. The plants capitola were covered by brown paper bags at the end of the pollination (R<sub>6</sub>) to prevent bird damages.

On physiological maturity, plants were harvested near the base and, subsequently, plant height was measured and then separated into leaf, stem, capitulum, and seed. Leaf surface area was also measured using leaf area meter (Delta-T Leaf Area Meter; Delta-T Devices, Cambridge, UK) at the flowering stage. All samples were dried up at 75°C and leaves dry weight was recorded.

### Determination of Cellular Fatty Acid Patterns, Oil, and Protein Contents

The sunflower seeds oil and protein were evaluated using an Inframatic 8620 (Percon, Germany). Seeds oil was extracted based on Folch *et al.* (1957) protocol. Extracted fatty acids were transformed to their methyl esters (FAME) using the Metcalf *et al.* (1966) method, and were determined using a gas chromatography (Unicam 4600) equipped with a FID detector. A fused silica capillary column BPX70 (30 m×0.22 mm id) with a 0.25 µm film thickness (SGE) was used as the stationary phase and 0.2 µl of the FAME sample was injected into the chromatograph using a micro syringe. Helium was the carrier gas with a head pressure of 18 psi and injector and detector temperatures were adjusted at 250 and 300°C, respectively. The oven temperature was programmed to 160°C for 5 min and subsequently increased by 2.0°C/min to 200°C and held at that for 40 minutes. The FAME samples were identified through comparison of standards Aldrich or Sigma and the closest match to the retention time data and mass spectra. The fatty acid patterns were calculated and evaluated from the total identified fatty acids.

**Table 2.** Total nitrogen in the soil of the experimental field in each fertilization treatment before planting in the second year (2008)<sup>a</sup>.

Treatments	F <sub>1</sub>		F <sub>2</sub>		F <sub>3</sub>		F <sub>4</sub>		F <sub>5</sub>	
	I <sub>1</sub>	I <sub>0</sub>	I <sub>1</sub>	I <sub>0</sub>	I <sub>1</sub>	I <sub>0</sub>	I <sub>1</sub>	I <sub>0</sub>	I <sub>1</sub>	I <sub>0</sub>
Total nitrogen (%)	0.11	0.11	0.10	0.07	0.10	0.11	0.10	0.10	0.11	0.11

<sup>a</sup> The numbers are the means of three replications and for symbols definitions refer to text.



## Statistical Analysis

A split plot layout within randomized complete block design with three replications was used for analysis. Fertilizer treatments were in the main plots and PGPR treatments were in the sub plots. The main and interactional effects of the treatments were determined by analysis of variance (ANOVA) using the GLM procedure generated by SAS statistical software (SAS Institute 2002). Due to non-homogeneous error in Bartlett's test for some traits such as plant height, oil yield, biomass, arachidonic, linolenic and stearic acids, the data was analyzed separately for each year and the rest were combined.

## RESULTS

As Figure 1 shows, temperature was generally similar in both growing seasons, while natural rainfall varied between the 2007 and 2008 seasons. Total rainfall during the growing season (July-September) was 68.4 mm in 2007 and 1.3 mm in 2008.

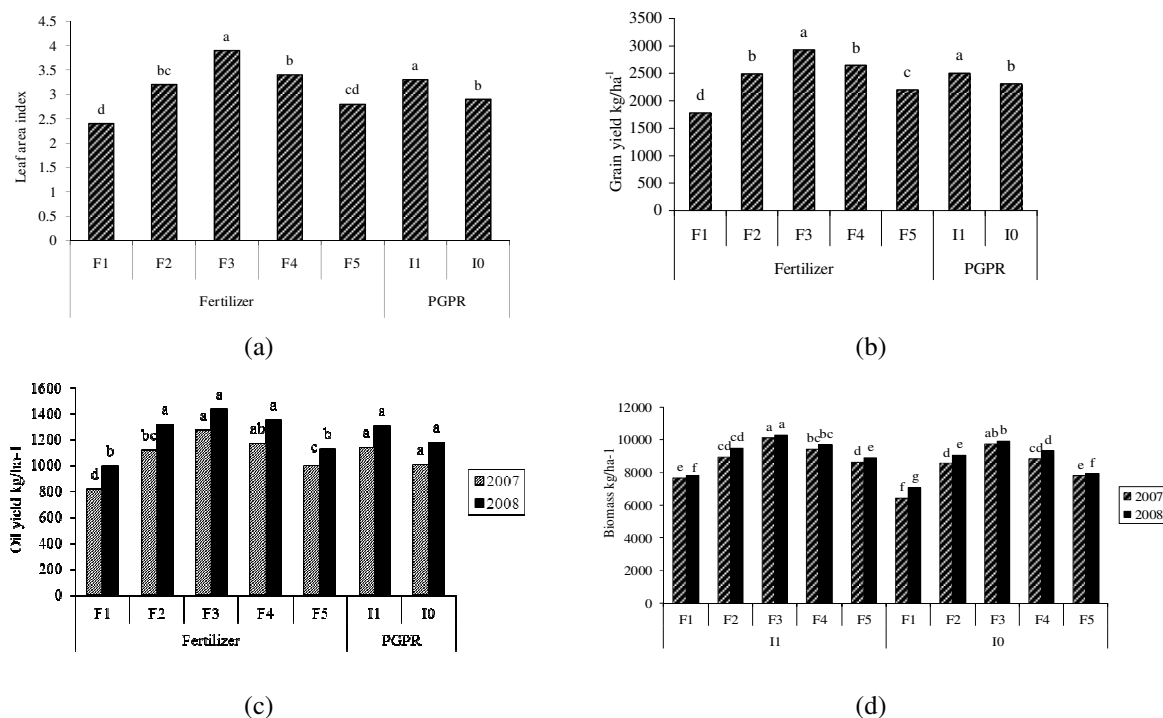
The main effects of fertilizer and PGPR factors were significant for leaf area index (LAI), plant height, and grain productions (Table 3). Averaged over both years, the integrated fertilization systems i.e., F<sub>3</sub>, F<sub>4</sub> and F<sub>2</sub>, respectively, and inoculating seeds with PGPR appear to have had a positive and significant effect on the LAI, plant height, grain yield, oil yield, and biomass (Figures 2; Table 4). As Figure 2-c and 2-d demonstrate, oil yield, and biomass are higher in 2008 than 2007. Inoculated plants recorded significantly higher biomass over the uninoculated (Figure 2-d).

Protein content was influenced by different nutrient sources, as depicted in Table 4. Significantly (P < 0.05) highest protein content was recorded in the treatment containing 50% FM. Bacterial inoculation significantly (P < 0.05) improved protein content as compared to uninoculated treatments. Oil content did not follow the same trend as protein content in terms of

**Table 3.** Summary of F significance from combined analysis of variance of treatments (fertilization systems and PGPR) in terms of biomass, grain and oil yields, oil, protein contents and fatty acids<sup>a</sup>.

SOV	DF	Mean square												
		Palmitic acid	Stearic acid	Oleic acid	Linoleic acid	Linolenic acid	Arachidonic acid	Biomass	Grain yield	Protein	Oil yield	Leaf area index	Height	
Year (Y)	1	1.1*	13.1**	0.5 <sup>ns</sup>	5.3 <sup>ns</sup>	0.11**	0.2*	1865603.1 <sup>ns</sup>	733057.1 <sup>ns</sup>	28.3*	97.5**	438750.1 <sup>ns</sup>	0.53 <sup>ns</sup>	770*
Fertilizer (F)	4	0.5**	1.5**	9.2**	31.6**	0.0008 <sup>ns</sup>	0.03*	13356270.8**	2320009.4**	12.4**	36.8**	372735.6**	4.21**	174 <sup>ns</sup>
Y×F	4	0.01 <sup>ns</sup>	0.3**	3.3*	2.8 <sup>ns</sup>	0.0001 <sup>ns</sup>	0.01*	70955.9 <sup>ns</sup>	5653.1 <sup>ns</sup>	0.04 <sup>ns</sup>	0.6 <sup>ns</sup>	1911.2 <sup>ns</sup>	0.05 <sup>ns</sup>	52*
Inoculation (I)	1	3.8**	1.8**	16.2**	41.2**	0.0004 <sup>ns</sup>	0.002 <sup>ns</sup>	5950860.6**	572717.4**	15.4**	24.7**	243913.9**	2.84**	454**
F×I	4	0.04 <sup>ns</sup>	0.14*	0.6 <sup>ns</sup>	4.4**	0.003**	0.01**	237698.6*	10651.5 <sup>ns</sup>	0.35 <sup>ns</sup>	0.6 <sup>ns</sup>	3376.4 <sup>ns</sup>	0.04 <sup>ns</sup>	2 <sup>ns</sup>
I×Y	1	0.6**	0.07 <sup>ns</sup>	0.1 <sup>ns</sup>	2.9*	0.0005 <sup>ns</sup>	0.000001 <sup>ns</sup>	51674.2 <sup>ns</sup>	3872.1 <sup>ns</sup>	0.05 <sup>ns</sup>	0.4 <sup>ns</sup>	67.3 <sup>ns</sup>	0.06 <sup>ns</sup>	109*
Y×F×I	4	0.07 <sup>ns</sup>	0.1*	0.8 <sup>ns</sup>	1.3*	0.0003 <sup>ns</sup>	0.01**	49756.4 <sup>ns</sup>	4097.5 <sup>ns</sup>	0.25 <sup>ns</sup>	0.2 <sup>ns</sup>	1072.2 <sup>ns</sup>	0.16*	18 <sup>ns</sup>

<sup>a</sup> ns: Not significant at the 0.05 probability level; \* Significant at the 0.05 probability level, \*\* Significant at the 0.01 probability level.



**Figure 2.** Leaf area index (a) Grain yield (b) Oil yield (c) and Biomass as influenced by two-way interaction (d) as influenced by different fertilization systems and PGPR in the combined analysis of two years. Significant differences according to the *LSD* at  $P < 0.05$  have been indicated with different letters. F<sub>1</sub>: 100% farmyard manure (FM) 48 t/ha<sup>-1</sup>; F<sub>2</sub>: 75% FM+25% chemical; urea 240 kg/ha<sup>-1</sup>; F<sub>3</sub>: 50% FM+50% chemical; F<sub>4</sub>: 25% FM+75% chemical; F<sub>5</sub>: 100% chemical; I<sub>1</sub>: Inoculating, and I<sub>0</sub>: Non-inoculating with PGPR.

integrated fertilization treatments (Table 4). The highest oil content (51.1%) was achieved in FM application alone (F<sub>1</sub>), whereas the lowest (46.3%) was obtained in the integrated treatment of 50% FM (Table 4). The oil content was higher in inoculated

plants than that in the uninoculated ones (Table 4).

The oil fatty acid composition showed significant differences in relation to the treatments studied in the fertilization systems with or without inoculation (Tables

**Table 4.** Some oil compositions (% of total fatty acids), oil and protein contents and plant height as influenced by different fertilization systems and PGPR in either annual or combined analysis of the two years<sup>a</sup>.

PGPR	Fertilizer	Palmetic acid	Oleic acid	Oil content (%)	Protein content (%)	Height (cm)	
						2007	2008
	F <sub>1</sub>	5.82c	39.8a	51.1a	18.3d	148.5d	148.8a
	F <sub>2</sub>	6.03b	39.1ab	49.0b	19.1c	158.0bc	160.5a
	F <sub>3</sub>	6.05b	37.7c	46.3d	20.9a	166.0a	163.3a
	F <sub>4</sub>	6.17b	37.9c	47.6c	20.2b	163.1ab	160.8a
	F <sub>5</sub>	6.40a	38.5bc	48.4bc	19.9b	154.6cd	157.3a
I <sub>1</sub>		5.80b	39.1a	49.1a	20.2a	160.2a	163.3a
I <sub>0</sub>		6.30a	38.1b	47.8b	19.2b	155.9b	155.3b

<sup>a</sup> Means within each column followed by the same letter(s) are not significantly different according to the *LSD* ( $P < 0.05$ ). For symbols definitions refer to text.



3-5). High amounts of unsaturated fatty acids, mainly linoleic and oleic acids, with more than 48 and 38%, respectively, were found in the sunflower oil under different treatments. The highest value for oleic acid (39.8%) was found in FM treatment alone (F<sub>1</sub>) and the lowest (37.7%) in 50% FM application. This fatty acid increased about 2.56% with bacterial inoculation as compared to non-inoculation (Table 4). The FM application along with chemical fertilizer in ratio of 50: 50 (F<sub>3</sub>) resulted in the highest linoleic acid for both PGPR treatments (Table 5). The saturated fatty acid (palmitic and stearic acid) concentrations tended to increase under chemical treatments (Tables 4 and 5). Other fatty acid components in the sunflower seed oil were linolenic and arachidonic acid, which did not show a clear trend under the studied treatments.

## DISCUSSION

An increase in plant ability to absorb nutrients causes the increase of growth and photosynthesis, therefore, an increase in plant leaf area in integrated systems (Shah and Ahmad, 2006). LAI is an expression of the canopy density of a crop population, and

plays an important role in yield formation. As a rule, the crop yield increases until an optimal LAI value is reached (Liu *et al.*, 2008). On the other hand, bacteria can directly affect plant growth in a useful way by increasing nitrogen absorption, the synthesis of phyto-hormones, and dissolving minerals (Herman *et al.*, 2008). Access to more nitrogen in soil, the increase of absorption and more photosynthesis by plants are the factors that result in plant height increase in the integrated treatments. In agreement with the present experiment, height increases of plants due to the integrated application of chemical and organic fertilizers have been reported by other researchers on wheat (Ramshwar and Singh, 1998) and maize (Clark *et al.*, 1999). In addition, Zahir *et al.* (2000) reported an increase of 8.5% in height of maize whose seed had been inoculated with *Azotobacter* and *Pseudomonas*.

Biomass is an important parameter to determine the photosynthetic activity of a crop. However, the increasing access of plants to nutrients, especially nitrogen, via the combined use of organic and chemical fertilizers cause an increase in growth and photosynthesis and also in the leaf area, which are the main factors underlying variation in biomass in integrated systems

**Table 5.** Contents ((% of total fatty acids) of linoleic acid, stearic acid, linolenic acid and arachidonic acid as influenced by two-way interaction between different fertilization systems and PGPR in either annual or combined analysis of two years.

PGPR	Fertilizer	Linoleic acid	Stearic acid		Linolenic acid		Arachidonic acid	
			2007	2008	2007	2008	2007	2008
I <sub>1</sub>	F <sub>1</sub>	48.4de	3.23c	4.47de	0.29bc	0.20g	0.86bcd	0.93a
	F <sub>2</sub>	49.2c	3.23c	4.35e	0.33ab	0.25a	0.87bcd	0.76f
	F <sub>3</sub>	52.6a	3.43bc	4.27e	0.31abc	0.22e	0.86bcd	0.66k
	F <sub>4</sub>	52.1a	3.53bc	4.67cd	0.33ab	0.23d	0.97ab	0.79e
	F <sub>5</sub>	50.9b	4.33a	4.99ab	0.31abc	0.21f	0.90abcd	0.74g
I <sub>0</sub>	F <sub>1</sub>	47.8e	3.17c	4.66cd	0.34a	0.25b	1.01a	0.85c
	F <sub>2</sub>	48.3e	3.80b	4.86bc	0.32abc	0.23d	0.95abc	0.89b
	F <sub>3</sub>	51.8a	3.76b	4.63d	0.30abc	0.23d	0.83cd	0.72i
	F <sub>4</sub>	49.1cd	4.43a	4.86bc	0.28c	0.22e	0.82d	0.81d
	F <sub>5</sub>	48.1e	4.66a	5.14a	0.33abc	0.24c	0.91abcd	0.67j

Means within each column followed by the same letter(s) are not significantly different according to the LSD ( $p < 0.05$ ).

For symbols definitions refer to text.

(Ali *et al.*, 2004; Basu *et al.*, 2008). In both years of the present study, the highest biomass was observed in F3 treatment with bacterial inoculation (10,117.9 and 10,280.0 kg ha<sup>-1</sup>). According to the results of other researchers such as Shata *et al.* (2007), we can conclude that the presence of manures and biological fertilizers increases nitrogen absorption from chemical fertilizers. In designing effective short- and long-term management strategies, the use of effective microorganisms along with organic/inorganic materials is an effective technique for stimulating the supply and release of nutrients from these nutrient sources (Khaliq *et al.*, 2006). A significant correlation was seen between biomass and grain and oil yields ( $r=0.90^{**}$  and  $0.87^{**}$ , respectively). This proves that every factor augmenting the biomass will increase the grain and ultimately the oil yield due to higher photosynthesis rate and increased production of photosynthates. As the results show, oil yield and biomass in the second year were higher than the first year, which can be attributed to the long term applications of chemical fertilizer combined with organic manure. Indeed, this practice plays an important role in improving soil properties and continually increasing yield of crops (Ming-Gang *et al.*, 2008).

Integrated fertilization systems including F<sub>2</sub>, F<sub>3</sub> and F<sub>4</sub> produced about 28.7, 39.2, and 32.8% more grain yield compared to F<sub>1</sub> and 12.0, 25.0, and 17.0% compared to F<sub>5</sub>, respectively (Figure 2-b). The combined use of inorganic fertilizers and organic manures can enhance the inherent nutrients supplying capacity of the soil with respect to both macro- and micronutrients (Stevenson *et al.*, 1998; Hao and Chang, 2002), and also improve the physical properties of the soil, which promote better rooting, higher nutrient uptake by the crop, and increase in leaf area, height, and, therefore, the dry matter production and grain yield. The lowest grain yield was achieved in F<sub>1</sub> nutritional system (Figure 2-b), probably due to the fixation of mineral nitrogen by organic fertilizers, especially in the early

growth stages of the plant, and also the long term and non-immediate effects of organic nutrients on crop yield. Soil microorganisms are very important in the biogeochemical cycles of both inorganic and organic nutrients in the soil and in the maintenance of soil health and quality (Jeffries *et al.*, 2003). Daly and Stewart (1999) reported that the application of PGPR to onion, pea, and sweet corn increased yields by 29, 31 and 23%, respectively.

Seeds from F<sub>3</sub> treatment had the highest protein content (20.9%). By decreasing nitrogen wastage, the integrated fertilization systems can provide more nitrogen for the crop, and increase protein content (Kheir *et al.*, 1991). The protein content of inoculated plants was more than non-inoculated ones (Table 4). This result was consistent with the finding of Ram Rao *et al.* (2007). This might be due to *Azotobacter*, which provides biologically fixed nitrogen for plants, and secretes beneficial growth promoting substances like IAA, GA, kinetin, riboflavin, and thiamine (Malik *et al.*, 2005). In the present experiment, a negative correlation existed between oil and protein contents. In oil crops, an increase in oil content is generally associated with a decrease in protein content as a result of a dilution effect (Aguirrezábal *et al.*, 2009). Basu *et al.* (2008) showed that applying farmyard manure was effective in improving the growth, nitrogen fixation, yield and kernel quality like oil content, mineral composition, and hydration coefficient of peanut. Bacterial inoculation had a significant effect on oil content (Table 3). Shehata and EL-Khawas (2003) reported a significant increase in sunflower oil content with the use of the bacterial fertilizer. Considering the fact that the *Azotobacter* and *Azospirillum* are nitrogen fixing bacteria, their function depends on the availability of nitrogen in the environment. In case of an abundance of mineral nitrogen in soil (F<sub>3</sub>), these bacteria may become nitrogen consumers and reduce the available nitrogen for plants.



Oil yield followed the same trend as seed yield (Figure 2-c). This could be attributed to grain yield and there was a highly significant correlation between them ( $r= 0.97^{**}$ ). The application of chemical fertilizers combined with organic manures was propitious to coordinate the balance of carbon and nitrogen pools, thereby increasing the system productivity (Kaur *et al.*, 2007).

In this study, linoleic acid was raised in response to the combined fertilization system while the oleic acid was reduced. These results agree with Munir *et al.* (2007) reports. The presence of bacteria can have positive effects on plant growth, increase in unsaturated fatty acids, and decrease in saturated fatty acids (Shehata and EL-Khawas, 2003), which are consistent with the results of this study. In fact, saturated fatty acids decrease while unsaturated fatty acids increase with the increase in the amount of nitrogen (Kheir *et al.*, 1991; Khaliq, 2004).

## CONCLUSIONS

Based on the results of this research, the FM application alone or in combination with bacterial inoculation can result in the highest oil content (up to 51%) and oleic acid (up to 40%). The FM application along with chemical fertilizer in 50:50 ratio with or without bacterial inoculation has been shown to increase oil yield between 1,276 and 1,436 kg ha<sup>-1</sup> in sunflower culture. Overall, the results suggest that sunflower productivity and seed oil quality can be improved using integrated fertilization systems.

## ACKNOWLEDGEMENTS

The authors would like to thank the financial support of the project by Tarbiat Modares University, Iran.

## REFERENCES

1. Aguirrezábal, L., Martre, P., Pereyra-Irujo, G., Izquierdo, N. and Allard, V. 2009. Management and Breeding Strategies for the Improvement of Grain and Oil Quality. In: "Crop Physiology", (Eds.): Sadras, O. and Calderini, D.. Academic Press, London, PP. 387-411.
2. Ali, H., Randhawa, S. A. and Yousaf, M. 2004. Quantitative and Qualitative Traits of Sunflower (*Helianthus annuus* L.) as Influenced by Planting Dates and Nitrogen Application. *Int. J. Agric. Biol.*, **6**: 410-412.
3. Basu, M., Bhadoria, P. B. S. and Mahapatra, S. C. 2008. Growth, Nitrogen Fixation, Yield and Kernel Quality of Peanut in Response to Lime, Organic and Inorganic Fertilizer Levels. *Biores. Technol.*, **99**: 4675-4683.
4. Chandrasekar, B. R., Ambrose, G. and Jayabalan, N. 2005. Influence of Biofertilizers and Nitrogen Source Level on the Growth and Yield of *Echinochloa frumentacea* (Roxb.) Link. *J. Agr. Technol.*, **1**: 223-234.
5. Clark, S., Klonsly, K., Livingston, P. and Temple, S. 1999. Crop-yield and Economic Comparisons of Organic, Low-input and Conventional Farming Systems in California's Sacramento Valley. *Am. J. Alternative Agr.*, **14**: 109-121.
6. Daly, M. J. and Stewart, D. P. C. 1999. Influence of "Effective Microorganisms" (EM) on Vegetative Production and Carbon Mineralization: A Preliminary Investigation. *J. Sustain. Agr.*, **14**: 15-25.
7. Folch, J., Less, M. and Sloane-Stanley, G. H. 1957. A Simple Method for the Isolation and Purification of Total Fatty Acids from Animal Tissues. *J. Biol. Chem.*, **226**: 497-507.
8. Hailu, S., Seyoum, T. and Dechassa, N. 2008. Effect of Combined Application of Organic-P and Inorganic-N fertilizers on Yield of Carrot. *Afr. J. Biotechnol.*, **7**: 27-34.
9. Hao, X. and Chang, C. 2002. Effect of 25 Annual Cattle Manure Applications on Soluble and Exchangeable Cations in Soil. *Soil Sci.*, **167**: 126-134.
10. Helmy, A. M. and Ramadan, M. F. 2009. Agronomic Performance and Chemical response of Sunflower (*Helianthus annuus* L.) to Some Organic Nitrogen Sources and Conventional Nitrogen Fertilizers under Sandy Soil Conditions. *Grasas Aceites.*, **60**: 55-67.
11. Herman, M. A. B., Nault, B. A. and Smart, C. D. 2008. Effect of Plant Growth-promoting Rhizobacteria on Bell Pepper Production and Green Peach Aphid Infestation in New York. *Crop Prot.*, **27**: 996-1002.
12. Hussein, M. A., El Hattab, A. H. and Ahmed, A. K. 1980. Effect of Plant Spacing and



- Nitrogen Levels on Morphological Characters, Seed Yield and Quality in Sunflower (*Helianthus annuus* L.). *J. Agron. Crop Sci.*, **149**: 148-156.
13. Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K. and Barea, J. M. 2003. The Contribution of Arbuscular Mycorrhizal Fungi in Sustainable Maintenance of Plant Health and Soil fertility. *Biol. Fert. Soils*, **37**: 1-16.
  14. Kaur, T., Brar, B. S. and Dhillon, N. S. 2007. Soil Organic Matter Dynamics as Affected by Long-term Use of Organic and Inorganic Fertilizers under Maize-wheat Cropping System. *Nutr. Cycl. Agroecosys.*, **10**: 110-121.
  15. Khaliq, A. 2004. Irrigation and Nitrogen Management Effects on Productivity of Hybrid Sunflower (*Helianthus annuus* L.). PhD. Thesis, Department of Agronomy, University of Agriculture, Faisalabad, Pakistan.
  16. Khaliq, A., Abbasi, M. K. and Hussain, T. 2006. Effects of Integrated Use of Organic and Inorganic Nutrient Sources with Effective Microorganisms (EM) on Seed Cotton Yield in Pakistan. *Biores. Technol.*, **97**: 967-972.
  17. Kheir, N. F., Harb, E. Z., Moursi, H. A. and El-Gayar, S. H. 1991. Effect of Salinity and Fertilization on Flax Plants (*Linum usitatissimum* L.). II. Chemical Composition. *Bull. Faculty Agricultura, Univ. Cairo*, **42**: 57-70.
  18. Liu, X., Jin, J., Wang, G. and Herbert, S. J. 2008. Soybean Yield Physiology and Development of High-yielding Practices in Northeast China. *Field Crop Res.*, **105**: 157-171.
  19. Mahapatra, B. S. and Sharma, G. L. 1989. Integrated Management of Sesbania, Azolla and Urea Nitrogen in Low Land Rice under Rice±Wheat Cropping System. *J. Agr. Sci.*, **113**: 203-206.
  20. Malik, B. S., Paul, S., Sharma, R. K., Sethi, A. P. and Verma, O. P. 2005. Effect of Azotobacter Chroococcum on Wheat (*Triticum aestivum*) Yield and Its Attributing Components.
  21. Merrién, A. and Milan, M. J. 1992. *Physiologie du Tournesol*. CETIOM, Paris, 66 PP.
  22. Metcalf, L. C., Schmitz, A. A. and Pelka, J. R. 1966. Rapid Preparation of Methyl Esters from Lipid for Gas Chromatography Analysis. *Anal. Chem.*, **38**: 514-515.
  23. Ming-Gang, X., Dong-Chu, L., Ju-Mei, L., Dao-Zhu, Q., Yagi, K. and Hosen, Y. 2008. Effects of Organic Manure Application with Chemical Fertilizers on Nutrient Absorption and Yield of Rice in Hunan of Southern China. *Agr. Sci. China*, **7**: 1245-1252.
  24. Mohammad, S. 1999. Long-term Effect of Fertilizers and Integrated Nutrient Supply in Intensive Cropping on Soil Fertility, Nutrient Uptake a Yield of Rice. *Agr. Sci.*, **133**: 365-370.
  25. Munir, M. A., Malik, M. A. and Saleem, M. F. 2007. Impact of Integration of Crop Manuring and Nitrogen Application on Growth, Yield and Quality of Spring Planted Sunflower (*Helianthus annuus* L.). *Pak. J. Bot.*, **39**: 441-449.
  26. Ram Rao, D. M., Kodandaramaiah, J., Reddy, M. P., Katiyar, R. S. and Rahmathulla, V. K. 2007. Effect of VAM Fungi and Bacterial Biofertilizers on Mulberry Leaf Quality and Silk Worn Cocoon Characters under Semiarid Conditions. *Caspian J. Envir. Sci.*, **5**: 111-117.
  27. Ramshwar, C. and Singh, M. 1998. Effect of FYM and Fertilizaeon the Growth and Development of Maize (*Zea mays*) and Wheat (*Triticum aestivum*) in Sequence. *Indian J. Agr. Sci.*, **32**: 65-70.
  28. SAS Institute Inc. 2002. *The SAS System for Windows: Release 9.0*. Statistical Analysis Systems Institute, Cary, NC, USA.
  29. Schuster, W. H. 1993. *Die Züchtung der Sonnenblume (Helianthus annuus)*. Paul Parey Science Publ., Berlin and Hamburg, 188 PP.
  30. Shah, Z. and Ahmad, M. I. 2006. Effect of Integrated Use of Farm Yard Manure and Urea on Yield and Nitrogen Uptake of Wheat. *J. Agr. Biol. Sci.*, **1**: 60-64.
  31. Shata, S. M., Mahmoud, S. A. and Siam, H. S. 2007. Improving Calcareous Soil Productivity by Integrated Effect of Intercropping and Fertilizer. *Res. J. Agr. Biol. Sci.*, **3**: 733-739.
  32. Shehata, M. M. and El-Khawas, S. A. 2003. Effect of Two Biofertilizers on Growth Parameters, Yield Characters, Nitrogenous Components, Nucleic Acids Content, Minerals, Oil Content, Protein Profiles and DNA Banding Pattern of Sunflower Yield. *Pak. J. Biol. Sci.*, **6**: 1257-1268.
  33. Škorić, D. 1992. Achievements and Future Directions of Sunflower Breeding. *Field Crops Res.*, **30**: 231-270.
  34. Stevenson, F. C., Johnston, A. M., Beckie, H. J., Brandt, S. A. and Townley-Smith, L. 1998. Cattle Manure as a Nutrient Source of Barley and Oilseed Crops in Zero and Conventional Tillage Systems. *Can. J. Soil Sci.*, **78**: 409-416.



35. Suslow, T. V. and Schroth, M. N. 1982. Rhizobacteria of Sugar Beets: Effects of Seed Application and Root Colonization on Yield. *Phytopathol.*, **72**: 199-206.
36. Tandon, H. L. S. 1995. *Methods of Analysis of Soils, Plants, Waters and Fertilisers*. Fertilizers Development and Consultation Organisation, New Delhi, India. 144 PP.
37. Zheljzkov, V. D., Vick, B. A., Baldwin, B. S., Buehring, N., Astatkie T. and Johnson, B., 2009. Oil Content and Saturated Fatty Acids in Sunflower as a Function of Planting Date, Nitrogen Rate, and Hybrid. *Agron. J.*, **101**: 1003-1011.
38. Yang, S. M., Li, F. M., Suo, D. R., Guo, T. W., Wang, J. G., Sun, B. L. and Jin, S. L. 2005. Effect of Long-term Fertilization on Soil Productivity and Nitrate Accumulation in Gansu Oasis. *Scientia Agricultura Sinica*, **38**: 2043-2052. (in Chinese)
39. Zahir, A. Z., Abbas, S. A., Khalid, A. and Arshad, M. 2000. Substrate Depended Microbially Derived Plant Hormones for Improving Growth of Maize Seedling. *Pak. J. Biol. Sci.*, **3**: 289-291.

### افزایش کارایی کمی و کیفی آفتابگردان (*Helianthus annuus L.*) تحت سیستم‌های کوددهی تلفیقی

س. شوقی کلخوران، ا. قلاوند، س. ع. م. مدرس ثانوی، ع. مختصی بیدگلی، و پ. اکبری

#### چکیده

اثرات انواع کودها همچون کود دامی، اوره و ریزوباکتری‌های افزایش‌دهنده رشد گیاه (PGPR)، شامل جنس‌های ازتوباکتر و آزوسپریلیوم) روی عملکرد، اسیدهای چرب و میزان پروتئین و روغن آفتابگردان مورد بررسی قرار گرفت. یک آزمایش مزرعه‌ای در منطقه‌ای نیمه-خشک در ایران در سال‌های ۱۳۸۶ و ۱۳۸۷ شمسی اجرا گردید. آزمایش به صورت کرت خرد شده در قالب طرح بلوک کامل تصادفی در سه تکرار انجام شد. کرت‌های اصلی شامل پنج تیمار کودی و کرت‌های فرعی شامل دو تیمار PGPR بودند. نتایج نشان دادند که کوددهی تلفیقی در مقایسه با کوددهی ارگانیک یا شیمیایی به طور معنی‌داری سبب افزایش شاخص سطح برگ، ارتفاع بوته، تولید دانه، زیست‌توده، عملکرد روغن و مقدار پروتئین شد. بیشترین کمترین مقادیر روغن به ترتیب در تیمارهای F<sub>1</sub> (با کاربرد ۱۰۰ درصد کود دامی) و F<sub>3</sub> (۵۰ درصد کود دامی + ۵۰ درصد کود شیمیایی) به دست آمدند، ولی در مورد میزان پروتئین نتیجه برعکس بود. هنگامی که نسبت بالاتری از کود دامی به همراه PGPR استفاده شد، اسیدهای چرب اشباع (اسیدهای پالمیتیک و استئاریک) به طور معنی‌داری کاهش یافتند، در حالی که اسیدهای چرب غیراشباع (اسیدهای لینولئیک و اولئیک) افزایش یافتند. بیشترین میزان اسید لینولئیک (۵۲/۶٪) و اسید اولئیک (۳۹/۸٪) به ترتیب در تیمارهای F<sub>1</sub> و F<sub>3</sub> مشاهده شدند. همچنین تلفیق باکتریایی سبب افزایش شاخص سطح برگ، ارتفاع بوته، زیست‌توده، عملکردهای دانه و روغن و مقادیر روغن و پروتئین به ترتیب به میزان ۱۲٪، ۳/۷٪، ۷/۸٪، ۱۰٪، ۶/۵٪، ۵/۶٪ و ۵٪ گردید.