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Comparison of different nutritional levels and the effect of plant growth promoting rhizobacteria (PGPR) on the grain yield and quality of sunflower

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Abstract

An experiment was conducted in 2007 and 2008 to study the effect of organic, chemical and integrated nutritional levels and Plant Growth Promoting Rhizobacteria (PGPR) on the grain yield and quality traits of sunflower (*Helianthus annuus* L.). A total of five nutritional levels, including 100% organic (F1), 25% chemical and 75% organic (F2), 50% chemical and 50% organic (F3), 75% chemical and 25% organic (F4) and 100% chemical (F5), were utilised in the main plot, and two levels of PGPR inoculation (I0, I1) were established in sub-plots, respectively. The treatments were arranged in a split-plot design based on RCB with three replications. The height, leaf area index, biological yield, 1000-seed weight, number of seeds, head diameter, harvest index and qualitative properties (except for the oil content) were superior in the F3, F4, and F2 integrated nutritional levels compared to the completely organic (F1) and chemical levels (F5). In the F3 integrated level, the total increased seed yield (2924.9) was approximately 40% of the lowest seed yield for the F1 level (1777.1). According to the results for the yield, the order was F3>F4>F2>F5>F1 respectively. The highest and lowest contents of oil were observed in the F1 level (51.07) and F3 level (49.35), respectively. The results showed that inoculating the seeds with PGPR increased the qualitative and quantitative properties of sunflower significantly, as compared to the control treatment.

Keywords: Chemical fertiliser; Farmyard manure; Integrated fertiliser; Plant Growth-Promoting Rhizobacteria; Sunflower.
Abbreviations: PGPR- Plant Growth Promoting Rhizobacteria; I- Inoculation; F- Fertiliser, FYM- Farmyard Manure.

Introduction

Intensive farming practices, which produce high yields and quality, require the extensive use of chemical fertilisers that are both costly and create environmental problems. Therefore, there has been a recent resurgence of interest in environmentally friendly, sustainable and organic agricultural practice (Orhan et al., 2006). Such sustainable agricultural management practices are vital in the production of healthy food for humans and animals. In the soil organic cropping system, the intention is to replenish the nutrient status and organic matter content of the soil through the use of plant residues, farmyard manure (FYM) and plant rotation (Niemi et al., 2008). Manure has long 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 the capabilities and goals of each farm, while enhancing the soil quality, crop nutrition, and profit (Nowak et al., 1998). Manure management is defined as a decision-making process that aims to combine profitable agricultural production with minimal nutrient losses from manure, both in the present and in the future (Brandjes et al., 1996). The selection of manure management and treatment options increasingly depends on environmental regulations with regard to the prevention of pollution of the soil, water and air. For example, regulated reductions in ammonia emissions could influence housing management, the storage and treatment of manure, and the methods of land application (Westerman and Bicudo, 2005). One of the major essential elements for growth of plants is nitrogen, which is required in large quantities for plants growth, as it is a fundamental constituent of proteins and nucleic acids. The excessive use of chemical fertiliser is undesirable because (1) the production of chemical fertilisers is a costly process, (2) most of the energy for fertiliser production is provided by the consumption of non-renewable fossil fuels, and (3) considerable pollution is caused by both the production and use of mineral N-fertilisers, which is exacerbated by the relatively low efficiency of their uptake by the. In contrast, the application of beneficial microbial populations to the soil can enhance plant resistance to adverse environmental stresses, water and nutrient deficiency and heavy metal contamination (Wu et al., 2005). Plant growth-promoting rhizobacteria (PGPR) are beneficial to plants due to the increased nutrient acquisition, biocontrol (Walsh et al., 2001), plant hormone-like production, lowering of plant ethylene levels (Glick, 1995) and induction of systemic resistance (van Loon et al., 1998). Incorporation of organic matter (OM) in the form of FYM enhances the organic carbon level of the soil and has direct and indirect effects on soil properties and processes (Prakash et al., 2007). Soil organic carbon is one of the indices of sustainability of a production system under a set of management practices because organic matter enhances the soil quality by improving the soil structure, nutrient storage, and biological activity (Ghosh et al., 2003). It has been reported that sunflower gave a higher yield from a combination of organic manures with chemical fertiliser and biofertiliser (Shata et al., 2007), and the highest grain yield has been recorded in sunflower with the application of poultry manure, as compared to other organic manures (Munir et al., 2007). Different plants are relatively easy to cultivate using organic methods if enough nutrients are available due to the application of biofertiliser into the soil. However, to date, there have been no attempts to study the effects of PGPR, including N2-fixing bacteria, and different nutritional levels on the growth of sunflower for improving nitrogen management practices to maximise the grain yield production with no concomitant reduction in the oil content. Therefore, we studied the effect of two N2- fixing bacterial strains (*Azospirillum* and *Azotobacter*) on the growth of sunflower using organic, inorganic and integrated fertiliser levels with a focus on the reduction of chemical fertiliser consumption, system self-stability and a transition towards sustainable agriculture.

Results and discussion

According to the results of the Bartlett test, the χ^2 was significant (P<0.05) for the biological yield, number of seeds, harvest index (HI) and plant height. The effects of fertiliser and PGPR on the four under study traits (except for the leaf area index [LAI] and height) was significant (P<0.05) for the two continuous years. In contrast, the χ^2 was not significant (P<0.05) for the seed yield and head diameter, leaf area index, 1000-seed weight, oil content and protein content. The various nutritional levels and PGPR had a significant effect on all of the traits ($p \leq 0.01$), but the interaction effects between the PGPR and nutritional levels and their interaction effect on the year were not significant (P>0.05) for any of the traits (except for the LAI). In addition, the effect of the year was not significant except for two traits: the protein content and the oil content (Table 3). It should be noted that the significant results using the Bartlett test for some of the traits may be related to the temperature differences between the two years.

Vegetative growth

The integrated nutritional levels had a positive and significant effect on the leaf area index (Table 6). An increased ability for nutrient uptake caused an increase in growth and photosynthesis and, therefore, increased crop leaf area in the plots under integrated nutritional levels. These results are in agreement with those obtained by Beauchamp et al. (1986) and Shah and Ahmad (2006). The seeds inoculated with PGPR caused a significant increase in the LAI, as compared to the control treatment (Table7). It can, thus, be concluded that these bacteria directly affected the growth of the plants by increasing the nitrogen absorption, the synthesis of phyto-hormones and the dissolving of minerals (Herman et al., 2008). The F3 integrated nutritional level exhibited the greatest height value and was followed by the F1, F2, F4 and F5 levels, in that order (Table 4). The increased height under the integrated treatments could be attributed to a higher nitrogen content in the soil, an increased absorption of nitrogen and increased photosynthesis. Similar results due to the integrated application of chemical and organic fertilisers have been reported previously in wheat and maize (Clark et al., 1999). The PGPR treatment increased the crop height in comparison to the control treatment (Table 5). These results are in agreement with the findings in chilli and millet (Chandereskar et al., 2005). The integrated nutritional levels resulted in the highest biological yield, and the F3 level exhibited the highest biological yield among the other treatments. The biological yield of the F5 level was less than the integrated levels, and the F1 level exhibited the lowest biological yield (Table 4). As compared to the organic and chemical levels, the observed increases in the biological yield at the integrated levels may be attributed to the increases in the vegetative components (leaf area and height) and reproductive components (head weight, number of seeds and 1000-seed weight). As has been previously confirmed (Basu et al., 2008), a higher absorption of nutrients increases the growth and biochemical activities of the plant, resulting in an increase in the seed and biological yield of plant. The results for both years (2007 and 2008) indicated that the sunflower seeds inoculated with PGPR exhibit a better biological yield, as compared to the control treatment (Table 5). In this study, plant growth promoting rhizobacteria resulted in a higher dry matter per plant, which led to increased vegetative growth and, therefore, caused a more effective use of the incident radiation, increased photosynthesis and an increase in growth. Shata et al. (2007) have reported an increase of 15% in the biological yield for a 50% chemical fertiliser plus organic and bio fertiliser treatment.

Yield components

The F3 and F4 integrated nutritional levels exhibited the largest values for the head diameter, followed by the F5, F1 and F2 levels, respectively (Table 6). However, the smallest diameter was observed in the F2 level. The head diameter in the PGPR-inoculated treatments increased in comparison to the un-inoculated treatment (Table 7). The order for the value of the 1000-seed weight for the nutritional levels was determined to be F3>F4>F2>F5>F1, with the highest 1000-seed weight being recorded in the integrated nutritional levels (Table 6). This result is in agreement with previous results in canola plants. The seeds inoculated with PGPR had a higher value for the weight of 1000 seeds, as compared to the control seeds (Table 7). Biofertilisers increase the effect of organic and chemical fertilisers on agricultural production by increasing the activity of microbial biomass (Shata et al., 2007). Zahir et al. (1998) have reported that maize inoculated with *Azotobacter* and *Pseudomonas* bacteria increased their 1000-seed weight, and the grain yield was approximately 9.6% and 19.8%, respectively. Among the integrated nutritional levels, the F3 and F1 levels produced the highest and the lowest number of seeds, respectively (Table 4). The application of farmyard manure with chemical fertiliser increased the seed number per head due to the high activity of microorganisms, the ability of the plant to access more nitrogen in the soil and, possibly, higher nitrogen absorption and transfer of the nitrogen to the shoot, leading to an increase in the seed yield (Table 5). The results indicate that the inoculation of seeds with PGPR increased the number of seeds, when compared to the control treatment. The increase in the seed number and seed yield in the PGPR-inoculated treatments could be attributable to the exudation of plant growth regulators (PGRs), such as auxins (Fallik et al., 1989) and gibberellin (Lucangeli and Bottini, 1997) by *Azospirillum*, and also the exudation of auxin, gibberellin and cytokinin by *Azotobacter* (Martinez-Toledo et al., 1988). The integrated nutritional levels had the highest effect on the accumulation of seed dry matter, as compared to the effect of the organic and chemicals levels. Therefore, the F3, F4 and F2 nutritional levels ranked first, second and third for the

Table 1. Physical and chemical analysis of the soil in the study area

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

Table 2. Chemical analysis of the farmyard manure 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	2.52	0.56	1.25

Table 3. Effects of the year on the quality and quantity traits of sunflower by composite analysis

Year	Leaf area Index	1000-seeds weight (gr)	Head diameter (cm)	Seed yield (kg ha^{-1})	Protein content (%)	Oil content (%)
2007	3.07 a	60.72a	18.22a	2295.6 a	19.03 b	47.23 b
2008	3.26 a	63.19a	18.35a	2516.7 a	20.40a	49.78 a

columns with the same letter(s) did not differ significantly according to Duncan's multiple-range test ($P<0.05$)

harvest index, respectively (Table 4). The results of the analysis of variance verified that the PGPR had no significant ($P>0.05$) effect on the HI; however the HI of the inoculated sunflower plants was increased compared to that of the un-inoculated plants (Table 5).

Grain yield

The integrated nutritional levels resulted in the highest grain yield among the chemical and organic nutritional levels, and the F3 level had the highest yield compared to the F4 and F2 integrated nutrition levels (Fig. 1). This increase in yield for the integrated nutritional levels might be due to a better compatibility between the soil available nitrogen and the requirements of the plant, indicating that the mineral nitrogen content of the organic nutritional level was less than that of the chemical fertiliser at the beginning of growth when the demand is low, but, at the reproductive growth stages, the absorption continues for a longer time because of the continuous mineralisation process (Mooleki et al., 2004). Some reasons that lead to yield increases under integrated nutritional systems include the reduction of the soil bulk density, the increase of the water storage capacity and the granulated structure of the soil, the increase of microbial and enzymatic activities and the release of the nutrients in soil colloidal particles (Gryndler et al., 2008), as has been previously confirmed (Munir et al., 2007). The PGPR had a positive and significant effect on the sunflower grain yield, such that the yield of the plants under the inoculated treatment increased by approximately 8%, as compared to the un-inoculated yield (Fig. 1). This increase was possibly due to the existence of microbial communities in the soil or rhizosphere that promoted the growth of the plant through the cycling and availability of nutrients, increasing the health of the roots during the growth stage by competing with root pathogens and increasing the absorption of nutrients (Roesty et al., 2006). These results are in agreement those of others (Shehata and El-Khawas, 2003).

Protein and oil content

The protein content of sunflower seeds is considered an important property for applications in pressed seeds for farm animal feed. The investigation of a comparison of the means showed that the F3 level exhibited the highest protein content (20.97), followed by the F4 level (20.21), whereas the F1 level exhibited the lowest protein content (18.32) (Table 6). Applying integrated fertilisers provides more available nitrogen for the plant, possibly by preventing the dissipation of nitrogen due to the presence of farmyard manure, and, therefore, the protein content of the integrated fertiliser levels, which consisted of farmyard manure and chemical fertiliser, was higher compared to the other levels. In fact, the protein content of sunflower seeds increases as the access to nitrogen increases (Nanjundappa et al., 2001). Basu et al. (2008) have also reported that the highest protein content for integrated treatments was found with chemical and organic fertiliszer. In the present study, the protein content was improved by the inoculation of PGPR, as compared to the control treatment (Table 7). These results demonstrate the positive effects of rhizobacteria in improving the nutritional conditions of plants; as a result of the inoculation with bacteria in this study, the growth performance and physiological and metabolic activities of the plants increased (Ram et al., 2002). This result is in agreement with the results of other researchers (Sharma and Namdeo, 1999; Shehata and El-Khawas, 2003). The F1 organic nutritional level exhibited the highest seed oil content, followed by the F2 level (Fig. 2). Indeed, as nitrogen accessibility increases, the seed oil content decreases (Kasem and EL-Mesilhy, 1992). Munir et al. (2007) have investigated various fertiliser levels in sunflower and have shown that the integrated and control treatments exhibited the lowest and highest oil contents, respectively. Furthermore, the findings of Steer and Seiler (1990) have indicated that there is an inverse relationship between the amount of available nitrogen and the oil content. In the present study, the application of PGPR increased the oil content significantly compared to the control treatment (Fig. 2). Shehata and El-Khawas, (2003) have reported a

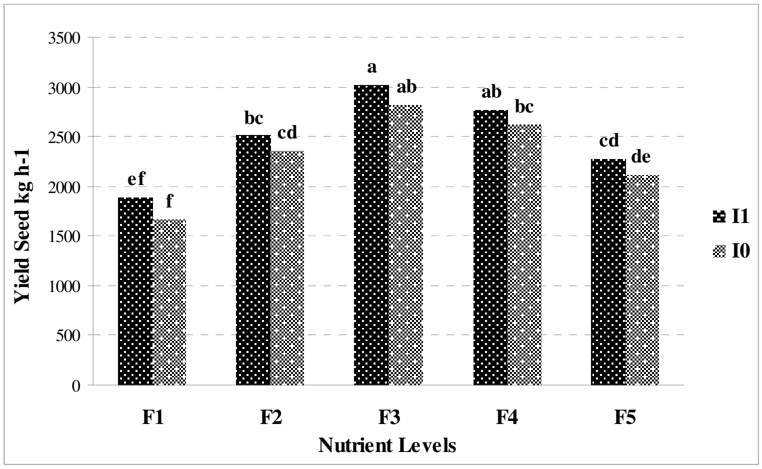

Fig 1. Influence of different nutrient levels (100% organic [F1], 25% chemical and 75% organic [F2], 50% chemical and 50% organic [F3], 75% chemical and 25% organic [F4] and 100% chemical [F5]) and PGPR (control [I0] and seed inoculation [I1]) on the yield during the 2007 and 2008 seasons by composite analysis.

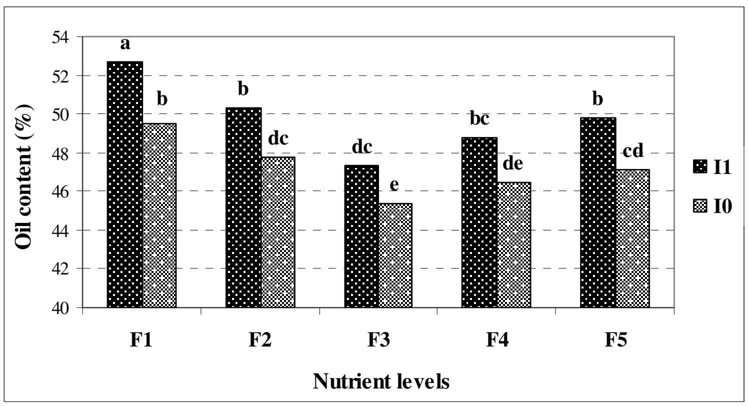

Fig 2. Influence of different nutrient levels (100% organic [F1], 25% chemical and 75% organic [F2], 50% chemical and 50% organic [F3], 75% chemical and 25% organic [F4] and 100% chemical [F5]) and PGPR (control [I0] and seed inoculation [I1]) on the oil content during the 2007 and 2008 seasons by composite analysis

significant increase in the sunflower oil content through the application of bio fertiliser, and Kumar (1994) has found that the oil yield increased by 10 to 40% by applying various *Azotobacter* strains to soybean plants.

Materials and methods

Location and plant material

This study was conducted at the experimental field area of the Agronomy Department, Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran (35° 44′N, 51° 10′E, altitude 1352 m), on sunflower (*Helianthus annuus*) in 2007 and 2008. Selected soil and manure properties are provided in Tables 1 and 2. The rows planted were 50 cm apart, and the plant density was 10 plants per m^2. Sunflower seeds from the Alestar cultivar were sown in May, and the seedlings were thinned at the 2-4-leaf stage. The crop exhibited no symptoms of insect/pest attack or disease; therefore, no extra protection management was adopted. The physiological maturity of sunflower was estimated visually by the change from a green to a yellow coloration of the backs of the heads. The seed yield and yield components were determined at harvest time in October by harvesting of middle rows of each plot after eliminating the margin effect. To determine the seed oil and the protein contents, ground seed samples were assayed using an Inframatic 8620 instrument (Perten Instruments, Inc, IL) that functions based on infrared spectrometry.

Experimental design

The experimental treatments were arranged in a split-plot design based on a randomised complete block design with three replications.Five levels of fertilisation were located in the main plots, as follows: 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); and F5, (100% N). In addition, the following two levels of biofertiliser were randomised to the sub-plots: I0, no biofertiliser (control) and I1, (inoculation with *Azospirillum* and *Azotobacter*).

Fertiliser and microbial inocula

All of the FYM was applied at a sowing rate of 32 tons ha^{-1}, and the nitrogen was applied at a rate of 260 $kg.ha^{-1}$ as urea in the two split plots; (½ N at the first irrigation and ½ N at

Table 4. Effects of different nutrient systems on selected quality and quantity traits of sunflower during the 2007 and 2008 seasons

Nutrition Levels	2007 Height (cm)	2007 Number of Seeds in Plant	2007 Harvest Index (%)	2007 Biological Yield (kg ha^{-1})	2008 Height (cm)	2008 Number of Seeds in Plant	2008 Harvest Index (%)	2008 Biological Yield (kg ha^{-1})
F1	148.5d	742.1 c	23.6 c	7020.7 c	148.5 a	748.4 d	25.4 b	7455.0 c
F2	158.0 bc	792.8 bc	26.9 ab	8732.2 ab	160.5 a	824.7 c	28.3 ab	9267.5 ab
F3	166.0 a	875.5 a	28.5 a	9917.9 a	163.3 a	922.1 a	29.9 a	10103.8 a
F4	163.1 ab	813.7 b	27.7 ab	9107.8 ab	160.8 a	867.8 b	28.9 ab	9516.7 ab
F5	154.6 cd	776.3 bc	25.9 bc	8203.5 bc	157,3 a	774.0 d	27.1 ab	8402/5 bc

columns with the same letter(s) did not differ significantly according to Duncan's multiple-range test ($P<0.05$)

Table 5.: Effects of PGPR on selected quality and quantity traits of sunflower during the 2007 and 2008 seasons

PGPR	2007 Height (cm)	2007 Number of seeds in Plant	2007 Harvest Index (%)	2007 Biological Yield (kg ha^{-1})	2008 Height (cm)	2008 Number of Seeds in Plant	2008 Harvest Index (%)	2008 Biological Yield (kg ha^{-1})
I1	160.2a	824.0 a	26.7 a	8940.7a	163.33 a	869.2a	28.58a	9403.8a
I0	155.9 b	776 b	26.3 a	8252.1 b	155.32 b	813.33b	28.27a	8887.3b

columns with the same letter(s) did not differ significantly according to Duncan's multiple-range test ($P<0.05$)

Table 6. Effects of nutrient levels on selected quality and quantity traits of sunflower by composite analysis

Nutrition Levels	Leaf Area Index	1000-Seed Weight (gr)	Head Diameter (cm)	Protein content (%)
F1	2.41d	55.95c	17.38 b	18.32d
F2	3.21bc	62.07b	18.07b	19.14c
F3	3.97a	67.12a	19.18a	20.97a
F4	3.42b	63.67b	18.90a	20.21b
F5	2.83cd	60.94b	17.90b	19.92b

columns with the same letter(s) did not differ significantly according to Duncan's multiple-range test ($P<0.05$)

Table 7. Effects of PGPR on selected quality and quantity traits of sunflower by composite analysis

PGPR	Leaf Area Index	1000-Seed Weight (gr)	Head Diameter (cm)	Protein content (%)
I1	3.38 a	64.06a	18.78a	20.22a
I0	2.95 b	59.81b	17.80b	19.21b

columns with the same letter(s) did not differ significantly according to Duncan's multiple-range test (P<0.05)

the flowering stage). Urea was used as the source of the inorganic (N) fertilisers. The sunflower seeds were inoculated with biofertiliser (*Azospirillum* and *Azotobacter*, 10^8 cfu g^{-1}) obtained from (Mehr Asia Biotechnology Company, Tehran, Iran) and maintained for 30 min in the shade.

Data analysis

Analysis of variance was carried out using SAS (SAS release 9.0, 2002), and a Bartlett test was performed on all of the parameters. The comparison of main effects was investigated using Duncan's Multiple Range Test at 0.05% probability.

Conclusions

We conclude that the combined application of recommended doses of chemical fertiliser and FYM to sunflower is a better nutrient management option for the sustained productivity of crops, as compared to using chemical or organic options alone. In addition, the inoculation of seeds with PGPR increased the yield and yield components of the sunflower plants significantly, as compared to the control treatment. Collectively, our results indicated that the combination of integrated fertilisers, using organic and inorganic fertiliser, with PGPR improved the growth and quality of sunflower plants, with a reduction of the chemical fertiliser consumption to help preserve the environment.

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