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Response of Sunflower Yield and Phytohormonal Changes to *Azotobacter*, *Azospirillum*, *Pseudomonas* and Animal Manure in a Chemical Free Agroecosystem

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ABSTRACT

There are new trends in agriculture to move toward the low input systems with the lower application of chemical fertilizers. To reach this goal, different methods, such as the application of biofertilizers, may be used. So this experiment was conducted in 2010 at a research farm in Arak, Iran, in factorial in the form of a randomized complete block design with three replications and four factors: animal manure (M), Pseudomonas putida (P), Azotobacter chroococcum (A) and Azospirillum lipoferum (Z). Results indicated that manure significantly affected grain yield (P≤0.01); the highest grain yield was achieved in the interaction of manure × Azotobacter × Pseudomonas (4.556 ton/ha). Grain yield was not significantly affected by the microorganisms. Moreover, the four factors of the experiment significantly affected auxin, gibberellin and cytokinin content of plant. Overall, this experiment indicated that desirable yield can be achieved by the application of manure and biofertilizers, in a sustainable agriculture.

Keywords: biofertilizer, organic farming, PGPR, nutrition.

INTRODUCTION

Oil crops are known as the source of energy and protein. They are important in human and livestock nutrition and play vital role in the economy of some countries. One of the important oil crops is sunflower which contains 40-50% oil and 15-21% protein [1]. Obtaining high yield with good quality heavily relies on soil nutritional condition. Some soil microorganisms are effective on plant growth and are called plant growth promoting rhizobacteria (PGPR). Groups of these bacterial species can associate plants; they belong to *Azospirillum*, *Azotobacter* and *Pseudomonas* genus [3]. PGPR include variety of bacteria such as the symbiotic nitrogen fixing

bacteria, non-symbiotic (free-living) nitrogen fixing bacteria, and sulfur, potassium phosphorus solubilizing bacteria [4].

Azospirillum is one of the most important nitrogen fixing microorganisms in tropical regions. *Azospirillum* associates with many monocots such as wheat, rice, maize, sorghum, sugar cane, rye and other grasses, and with some dicots. *Azospirillum* is widely distributed around the world and the association is reported in many tropical to cold climates, however, it is more common in warmer climates [5, 23]. Ardakani et al. (2011 b) found the inoculating wheat seeds by *Azospirillum* significantly increased plant NPK uptake [15]. Yadav et al. (2011) also concluded that *Azospirillum* inoculation reduced the need for chemical N fertilizer [22].

Pseudomonas bacteria can produce plant growth regulators and can inhibit the activity of harmful microorganisms in the rhizosphere. *Pseudomonas* bacteria are gram negative bacteria which naturally inhibit soil. *Pseudomonas fluorescence* and *P. putida* are the most important beneficial species of *Pseudomonas* genus which has been under study for years. These two species are entitled fluorescent *Pseudomonas* and are characterized by the pigments which look fluorescent under UV ray [2]. Gen and Jordan (1993) represented that inoculating tomato with *Pseudomonas fluorescence* enhanced weight of top quality fruits from 5.6% to 9.6% [20]. Moreover, fruit size was increased by 11.1%, and unmarketable fruits were reduced to 12% (in treatment) from 23% (in the control).

Animal manure improves soil physico-chemical properties, enhances soil nutrients and organic matter content, improves soil pH and CEC and promotes the activity of soil organisms [8, 11, 19]. Ardakani et al. (2011 b) reported that manure application increased wheat P uptake by 23.53% [15]. Mujiyati and Supriyadi (2009) also concluded that application of manure significantly increased chili yield [17].

This experiment was conducted with the objective of evaluating the effect of biologic and organic sources of nutrients on sunflower yield and phytohormones content.

MATERIALS AND METHODS

This experiment was conducted in 2010 at a research farm 3 km north of Arak, Iran (49^o 42' E, 34^o 5' N and 1757m above the sea level). Soil at the test site was a clay loam (sand: 44%, silt: 26% and clay: 30%) with pH of 8.2. Other properties of the test site's soil are listed in Table 1.

Table 1. Properties of the test site's soil.

O.C (%)	T.NV. (%)	Total N (%)	K_{ava} (ppm)	P_{ava} (ppm)	Mn (ppm)	Cu (ppm)	Zn (ppm)	Fe (ppm)	EC (ds/m)
0.7	31	0.07	242	7.1	5.7	0.3	0.4	5.3	2.2

The experiment was conducted in factorial in the farm of a randomized complete block design with three replications. The four factors of the experiment included: animal manure (with 30 ton/ha, M_1 and without, M_0), *Azotobacter* (with 20 g/1 kg seed, A_1 and without, A_0), *Azospirillum* (with 20 g/1 kg seed, Z_1 and without, Z_0) and *Pseudomonas* (with 20 g/1 kg seed, P_1 and without, P_0).

To inoculate seeds with the microorganisms, seeds were mixed with the solution of water and sugar to make seeds a bit sticky. Then, the inoculants were added to seeds under shadow and remained for 10 minutes to allow seeds to swell. Finally, seeds were planted, first in the control

plots to make sure they are non-inoculated. According to the standards, the population of the bacteria was 10^8-10^9 live cells/gram.

At the early flowering stage, samples were taken to measure auxin, gibberellin and cytokinin content. At the library, a Unicam HPLC was used for hormones extraction in isocratic method. Auxin and cytokinin were extracted in a C18-HiQsil column (5 µm × 4.6 mm × 25 cm) and gibberellin was extracted in a Zarbax S18 (3.5 µm × 2.1 mm × 15 cm). The standard solutions were prepared for the hormones as 1 g/L in 20% methanol. Then, formic acid (1%) was added to the solutions and samples were kept at 4°C. to extract auxin and cytokinin, 1 g of plan leaf was placed in 20 ml of a solution containing equal amount of methanol and deionized water and homogenized by the means of a homogenizer at 4°C. The obtained solution was centrifuged at 3000 round for 15 minutes. Then, the upper part of the solution was skimmed and injected in C18 column and eluted by 5 ml deionized water. After that, 3 ml of 80% methanol was added. The extracted solution was vaporized at room temperature by the means of a refrigerant. Finally, 1 ml of 20% methanol containing 1% formic acid was added to the remained material, 1 ml of 80% methanol was added again. This solution was used to determine the hormones content.

At the end, data were analyzed by SAS and means were compared by Duncan's multiple range test.

RESULTS AND DISCUSSION

Grain yield. Analysis of the variances indicated the significant effect of manure on grain yield (P≤0.01; Tables 2 and 3). This proves the ability of animal manure to meet plant nutritional requirements. Repeated application of manure reduces the pH of soils, especially the calcareous ones, resulting in the improvement of soil physico-chemical properties and increases the availability of nutrients to crops [13]. Most animal manures contain 0.01 to 0.05 available Zn which is a required nutrient for plants. Moreover, manure increases soil organic matters and natural chelates which increase soil Zn content.

Table 2. Analysis of the variances for the measured traits

SOV	df	Mean Squares (MS)			
		Grain Yield	Auxin	Gibberellin	Cytokinin
Replication	2	*	ns	ns	ns
Manure (a)	1	**	**	**	**
Azotobacter (b)	1	ns	**	**	**
Azospirillum (c)	1	ns	**	**	**
Pseudomonas (d)	1	ns	**	**	**
A×B	1	ns	ns	ns	ns
A×C	1	ns	ns	ns	ns
A×D	1	ns	ns	ns	ns
B×C	1	ns	ns	ns	ns
B×D	1	ns	ns	ns	ns
C×D	1	ns	ns	ns	ns
A×B×C	1	ns	ns	ns	ns
A×C×D	1	ns	ns	ns	ns
B×C×D	1	ns	ns	ns	ns
A×B×D	1	ns	ns	ns	ns
A×B×C×D	1	ns	ns	ns	ns
Error	30	622736.26	1396.09	868.50	1597.34
CV (%)	-	21.86	9.43	11.81	25.50

*ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.*

Ardakani et al. (2011 a) reported that manure application significantly increased wheat Fe, Mn, Zn and Cu uptake [14]. Sarajuoghi et al. (2011) represented that manure application significantly enhanced maize forage and grain yield by about 7% and 10%, respectively [16]. As manure contains high organic matters, it will improve soil physical conditions, resulting in better seed germination and emergence. Yazdani et al. (2008) found that application of different sources of organic matters improved soybean germination and emergence [18].

Table 3. Effects of manure (M), *Azotobacter* (A), *Azospirillum* (Z) and *Pseudomonas* (P) on the measured traits

Treatments	Grain Yield (kg/ha)	Auxin (mM/g of leaf)	Gibberellin (mM/g of leaf)	Cytokinin (mM/g of leaf)
M_0	3210.90b	372.50b	225.41b	132.48b
M_1	3976.60a	417.38a	271.50a	178.63a
A_0	3587.70a	371.13b	229.34b	135.74b
A_1	3633.10a	420.70a	269.57a	177.38a
Z_0	3725.30a	362.26b	219.12b	127.05b
Z_1	3495.60a	429.57a	279.79a	186.07a
P_0	3435.70a	366.45b	213.73b	132.12b
P_1	3770.50a	422.92a	282.21a	178.96a

Means in a column followed by the same letter are not significantly different.

Leaves auxin content. Results indicated that manure and the three microorganisms significantly affected sunflower leaves auxin content ($P \leq 0.01$; Table 2). However, none of the interactions had significant effect on this trait. The significant effect of treatments on auxin content can be because of the stimulation of tryptophan amino acid (auxin precursor) by the *Azotobacter* inoculation. Large groups of microorganisms are capable of producing phytohormones such as auxin, gibberellin, cytokinin and abscisic acid [7]. Some rhizobacteria including *Azospirillum*, *Agrobacterium*, *Pseudomonas* and *Erwinia* contribute to auxin production which is effective on the density and length of root hairs, elongation rate of lateral roots and generally, root system development [21].

Erturk et al. (2010) studied the effect of growth promoting rhizobacteria on Kiwi root system development and found that the bacteria were able of producing indole acetic acid (IAA), and the improved root system development was related to auxin production [24]. They suggested the possibility of using PGPR instead of the synthetic auxin (IBA). Furrani et al. (2008) reported that application of biofertilizers increased bean auxin content [10].

Leaves gibberellin content. Gibberellin is a phytohormone which is effective on plant growth and is produced in meristematic leaves, root ends and growing seeds. This hormone is freely transferred in xylem and phloem. Results of this experiment indicated that manure and the microorganisms significantly affected sunflower gibberellin content ($P \leq 0.01$; Table 2); the effect of the interactions was not significant. So many microorganisms can produce phytohormones such as gibberellin, which are naturally produced in root metabolism [7]. In plants body, growth and all other mechanisms are controlled by the chemical signals which are entitled phytohormones. One of the important mechanisms of plant growth improvement is the bacterial synthesis of phytohormones like indole acetic acid (IAA), cytokinin and gibberellin, and the increased nitrogen availability in soil. *Pseudomonas* can produce auxin, gibberellin and vitamins. Fluorescent *Pseudomonas* such as *P. putida* produces metabolites like auxin, gibberellin, ethylene and biotin, which are effective on plants and soil microorganisms' growth. Khalil (2009) reported the ability of *Azospirillum brasilense* and *Pseudomonas putida* to produce auxin and gibberellin, two vital phytohormones [6].

Leaves cytokinin content. Cytokinins are plant growth promoting substances which stimulate cell division. Cytokinins are produced in meristematic tissues, mainly in roots and then move upward to the shoots. However, roots are not the only source of cytokinins production. Results of this experiment represented that manure and the three microorganisms had significant effect of the plant cytokinin content ($P \leq 0.01$; Table 2). Although *Azotobacter* sp. are nitrogen fixing bacteria, they mainly affect plants growth by producing growth precursors. Barbara and Wong (1989) proved the production of cytokinin in *Azotobacter* culture medium. *Azotobacter* and *Azospirillum* are the most important PGPR and improve plants growth by the production of phytohormones in addition to the biological nitrogen fixation [9]. Fulchieri et al. (1993) attributed the improved maize growth and yield to the phytohormones production by *Azospirillum* bacteria [12].

CONCLUSION

Results of this experiment generally proved the significant effect of *Azotobacter*, *Azospirillum* and *Pseudomonas* on sunflower grain yield and phytohormones content. However, Manure application had only a significant effect on grain yield.

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