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Response of Agronomical Traits of Sunflower (*Helianthus annuus* L.) to Co-Inoculation with *Glomus intraradices* and *Pseudomonas fluorescens* under Different Phosphorus Levels

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ABSTRACT

This experiment was conducted in 2010 at the research field of Islamic Azad University, Takestan branch, Iran, to evaluate the effects of Mycorrhiza, Pseudomonas and chemical phosphorus on sunflower yield, quality and P uptake. The study was conducted in factorial in the form of a randomized complete block design with four replications and three factors: phosphorus (0, 50 and 100 kg/ha), Mycorrhiza (with and without inoculation) and Pseudomonas (with and without inoculation). Results indicated that phosphorus, Mycorrhiza and Pseudomonas significantly affected all the measured traits, except for the effect of Pseudomonas which was not significant on the weight of filled grains, oil yield and grain yield. The three-fold interaction had only a significant effect on plant dry weight. 100 kg P/ha × Mycorrhiza × Pseudomonas increased plant dry weight by 91.32% compared with the control.

Keywords: *Glomus intraradices,* oil crops, *Pseudomonas fluorescens*.

INTRODUCTION

Today, plant oils extracted from oil crops are the most important oils in food industries. Sunflower (*Helianthus annuus* L.), which is an important oil crop, contains high quality oil with high unsaturated fatty acid. Regarding the limited area of farmlands, the only way to increase agricultural production is the improvement of yield. Soil nutrients are key factor for plant growth. However, high soil pH makes minerals unavailable to plant roots. A common way to overcome these problems is the application of chemical fertilizers. However, chemical fertilizers are expensive and their application is associated with many health and environmental issues. A possible solution to these problems may be the biological ways.

Application of phosphate solubilizing microorganisms is an effective method of increasing the availability of some nutrients, especially P, in soils with high pH. Mycorrhizal symbiosis is the most common type of plant-microorganisms relations; about 90% of vascular plants form a symbiotic relation with Mycorrhizae [2, 21].

However, this common symbiosis is mainly disturbed by human activities such as uncontrolled application of phosphate containing fertilizers or chemical pesticides, especially fungicides. *Mycorrhiza* affects plant growth through different mechanisms. It improves nutrients and water uptake, produces phytohormones, alleviates the effects of environmental stresses, stabilizes soil aggregates, prevents root damages at the time of transplanting, increases plant tolerance to pathogens, facilitates the biological nitrogen fixation and stimulates the activity of some beneficial soil microorganisms [5, 16]. The improved absorption of water and nutrients in mycorrhizal plants is a function of the fungus hyphae. Mycelia, which are connected to plant roots, penetrate into soil pores and cracks; making higher volume of soil available to the plant and improve absorption. In fact, *Mycorrhiza* hyphae provide an extra absorptive surface for plant roots [13, 19]. Moreover, *Mycorrhiza* excretes phosphatases; solubilizing the fixed P in soil [12]. Ardakani and Mafakheri [20] reported that applying 90 kg P/ha without mycorrhizal inoculation gave significantly the same grain yield as 30 kg P/ha along with mycorrhizal inoculation. This finding proves that *Mycorrhiza* may be used instead of higher rates of chemical P fertilizers.

In addition to *Mycorrhiza*, some bacteria can also affect plant growth through direct and indirect mechanisms. In direct mechanisms, bacteria increase plant growth by producing phytohormones such as auxin and gibberellin, facilitating nutrients absorption, solubilizing the fixed minerals, and producing siderophores. In indirect mechanisms, the bacteria affect plant growth by increasing plants tolerance to pathogens or stresses [7, 15]. Asghar et al. [10] reported that plant growth promoting rhizobacteria increased brown mustard (*Brassica juncea* L.) growth. Son et al. [24] found that *Pseudomonas* spp inoculation increased the number and weight of nodules, grain yield and nutrient uptake in soybean. Finally, it can be concluded that biofertilizers are the main components of a sustainable organic production system, so this experiment was conducted to evaluate the effects of *Mycorrhiza*, *Pseudomonas* and different rates of chemical P fertilizer on sunflower yield, quality and P uptake.

MATERIALS AND METHODS

This experiment was conducted in 2010 at the research field of Islamic Azad University, Takestan branch, Iran (49^o 39' E, 36^o 3' N, 1325 m above the sea level). The soil type at the test site was loamy silt with the pH of 8.02. Other soil properties are listed in Table 1.

Table 1. Physico-chemical properties of the test site soil

EC (ds/m)	OC (%)	P_{ava} (ppm)	K_{ava} (ppm)	TNV	Saturation Percent
0.80	0.59	7.8	300	6	31

The experiment was conducted in factorial in the form of a randomized complete block design with four replications and three factors:

Mycorrhiza: with inoculation of *Glomus intraradices* (500 g fungi/3 kg sunflower seed; M_1) and without (M_0).

Pseudomonas: with inoculation of *Pseudomonas fluorescens* 187 (S_1) and without (S_0).

Chemical phosphorus fertilizer: 0 (P_0), 50 (P_1) and 100 (P_2) kg P/ha by the application of ammonium phosphate.

After field preparation, inoculated sunflower (*Helianthus annuus* L. cv. Armavirski) seeds were planted based on 120,000 plants/ha. To measure plant growth indices, six samplings were conducted with intervals of 10 days. After each sampling, the harvested samples were divided into leaf, stem and petiole, and were dried in a 70^{o}C oven for 48 h. The measured traits included grain yield, plant dry weight, oil yield, filled grains weight in plant, the number of filled grains in plant and P content. Data were analyzed using SAS and means were compared according to the Duncan's multiple range test.

RESULTS AND DISCUSSION

Results indicated the significant effect of phosphorus application on all measured traits (Table 2). 100 kg P/ha was the most effective P application rate and increased grain yield, oil yield and P content by about 24%, 43% and 28%, respectively, compared with the control (Table 3).

Table 2. Analysis of the variances for the measured traits

SOV	df	Mean Squares (MS)					
		P content	Number of filled grains in plant	Weight of filled grains in plant	Oil yield	Plant dry weight	Grain yield
Phosphorus (P)	2	*	**	**	**	**	**
Mycorrhiza (M)	1	*	*	*	*	*	*
Pseudomonas (S)	1	**	*	ns	ns	*	ns
P×M	2	ns	ns	ns	ns	*	**
P×S	2	ns	ns	ns	ns	ns	ns
M×S	1	ns	ns	ns	ns	ns	ns
P×M×S	2	ns	ns	ns	ns	*	ns
Error	33	0.16	69335.94	249.91	138984.89	102.74	650511.9
CV (%)	-	12.47	12.93	11.88	11.45	10.02	14.57

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

Table 3. The effects of phosphorus (P_0, 0; P_1, 50; P_2, 100 kg/ha), *Mycorrhiza* (with, M_1; without, M_0) and *Pseudomonas* (with, S_1; without, S_0) on the measured traits

Treatments	P content (%)	Number of filled grains in plant	Weight of filled grains in plant (g)	Oil yield (kg/ha)	Plant dry weight (g)	Grain yield (kg/ha)
P_0	0.28b	570.50b	28.45	1327.39b	502.88b	3461b
P_1	0.32ab	592.05b	30.88b	1511.83b	484.06b	3747b
P_2	0.36a	636.20a	35.53a	1898.71a	654.75a	4300a
M_0	0.29b	550.43b	27.89b	1347.73b	538.94b	3389b
M_1	0.34a	646.88a	35.35a	1804.64a	555.52a	4283a
S_0	0.29b	595.65b	31.41a	1521.45a	503.10b	3813a
S_1	0.34a	605.73a	31.83a	1621.16a	591.36a	3859a

Means in a column followed by the same letter are not significantly different at P≤0.05.

Phosphorus improves plants photosynthesis, resulting in higher assimilates production and consequently higher grain yield. These effects are because of the vital roles of P in plant. P is involved in many physiological processes and is essential especially in pollination and grain filling stages [2, 8]. In an experiment, it was concluded that application of 90 kg P/ha increased wheat grain yield by 18% [20]. They reported that 90 kg P/ha gave the highest harvest index (HI), representing that P is more effective on grain yield rather than vegetative growth. Magani and Kuchinda [11] also reported that increased P application rate enhanced grain yield and protein content of two cowpea cultivars.

Analysis of variances showed that mycorrhizal inoculation significantly affected all the measured traits (Table 2). Mycorrhizal inoculation increased grain yield, oil yield and P content by about 26%, 34% and 17%, respectively, compared with the control (Table 3).

Mycorrhiza symbiotic relation with plants is the most widespread plant strategy to secure more water and nutrients and to alleviate the damages of abiotic stresses [1]. The hyphae of *Mycorrhiza* penetrate into soil with lower energy than the plant roots do; increasing the total soil volume available for the plant roots. This facilitates water and nutrients absorption and enables plants to cope with the environmental stresses better. Moreover, *Mycorrhiza* is a phosphate solubilizing microorganism. The hyphae of the fungi release the fixed soil P and make it available to plant roots [2, 22].

To test the phosphate solubilizing ability of *Mycorrhiza*, Ardakani and Mafakheri [20] conducted an experiment and reported that application of 90 kg P/ha without mycorrhizal inoculation gave significantly the same wheat grain yield as 30 kg P/ha along with mycorrhizal inoculation. This finding proves the ability of *Mycorrhiza* to compensate for lower P application rates. In their experiment, mycorrhizal inoculation increased plant height by 2.69%, the number of kernels/panicle by 14.47% and 1000 kernels weight by 15%. Farzaneh et al. [17] found that *Mycorrhiza* increased chickpea growth by up to 43% (total dry matter) at maturity stage compared with the control. Neumann and George [6] also reported that mycorrhizal symbiosis increased nutrient uptake of drought stressed cowpeas.

Results of this experiment indicated that *Pseudomonas* inoculation significantly affected plant dry weight, the number of filled grains in plant and phosphorus content; the effect was not significant on rest of the measured traits (Table 2). The bacterium increased plant dry weight by 17.54%, the number of filled grains in plant by 1.7% and P content by 17.24% (Table 3).

Pseudomonas is a plant growth promoting rhizobacterium which improves plant growth through the direct and indirect mechanisms. The direct mechanisms include biological nitrogen fixation, phosphate solubilization, production of plant hormones such as auxin and cytokinin, enhancement of root development and improvement of other soil microorganisms' activity. The indirect mechanisms include the biological control of plant pathogens ([3, 4, 18]. In an experiment regarding the biological control ability of *Pseudomonas*, Klopper and Schroth [14] reported that *P. fluorescens* strain B10 inhibited barely wilt.

Mehran et al. [18] reported that *P. putida* significantly increased auxin, gibberellin and cytokinin content in sunflower; however, had no effect on grain yield. Khalil [9] also reported the ability of *P. putida* to produce phytohormones. Sandhya et al. [25] found that *Pseudomonas* sp. increased drought tolerance in maize seedlings by the synthesis of proteins, antioxidant, proline, amino acids and chlorophyll. Suslow and Schroth [23] observed that *P. fluorescens* inoculation increased sugar beet root / shoot dry weight by 20-25% in greenhouse condition, and by 6.1-8.6% in field conditions.

Results of this experiment also indicated that the two-fold and three-fold interactions had no significant effect on most of the measured traits (Tables 2, 4 and 5).

Table 4. The effects of the two-fold interactions of phosphorus (P_0, 0; P_1, 50; P_2, 100 kg/ha), *Mycorrhiza* (with, M_1; without, M_0) and *Pseudomonas* (with, S_1; without, S_0) on the measured traits

Treatments	P content (%)	Number of filled grains in plant	Weight of filled grains in plant (g)	Oil yield (kg/ha)	Plant dry weight (g)	Grain yield (kg/ha)
P_0M_0	0.26a	485.57a	24.17a	1064.19a	409.14c	2952b
P_0M_1	0.29a	654.71a	32.72a	1614.45a	596.62ab	3969ab
P_1M_0	0.31a	547.05a	26.91a	1276.04a	442.51bc	3271ab
P_1M_1	0.33a	631.62a	34.86a	1760.58a	525.73bc	4223ab
P_2M_0	0.33a	615.25a	32.56a	1640.17a	577.81abc	3946ab
P_2M_1	0.39a	655.70a	38.49a	2175.81a	731.58a	4656a
P_0S_0	0.27a	589.87a	28.36a	1220.81a	492.13a	3455a
P_0S_1	0.29a	551.69a	28.53a	1434.65a	513.74a	3466a
P_1S_0	0.27a	550.89a	28.47a	1361.01a	455.62a	3462a
P_1S_1	0.38a	631.89a	33.30a	1668.77a	512.62a	4032a
P_2S_0	0.32a	607.75a	33.74a	1768.49a	561.68a	4091a
P_2S_1	0.40a	664.39a	37.31a	2032.78a	747.71a	4510a
M_0S_0	0.29a	528.84a	27.41a	1285.38a	502.21a	3334a
M_0S_1	0.30a	573.18a	28.37a	1411.75a	574.00a	3445a
M_1S_0	0.30a	659.42a	35.44a	1770.44a	504.00a	4292a
M_1S_1	0.38a	635.86a	35.28a	1838.86a	608.72a	4273a

Means in a column followed by the same letter are not significantly different at $P\leq0.05$.

Table 5. The effects of the three-fold interactions of phosphorus (P_0, 0; P_1, 50; P_2, 100 kg/ha), *Mycorrhiza* (with, M_1; without, M_0) and *Pseudomonas* (with, S_1; without, S_0) on the measured traits

Treatments	P content (%)	Number of filled grains in plant	Weight of filled grains in plant (g)	Oil yield (kg/ha)	Plant dry weight (g)	Grain yield (kg/ha)
$P_0M_0S_0$	0.22a	456.47a	23.40a	940.21a	474.99bcd	2848.04a
$P_0M_0S_1$	0.29a	516.90a	24.94a	1194.51a	718.26ab	3057.08a
$P_0M_1S_0$	0.27a	747.21a	33.67a	1538.18a	509.15bcd	4085.64a
$P_0M_1S_1$	0.32a	581.65a	31.78a	1684.08a	309.12d	3853.72a
$P_1M_0S_0$	0.28a	535.66a	27.74a	1451.41a	467.94bcd	3376.36a
$P_1M_0S_1$	0.34a	559.69a	26.11a	1278.18a	417.09cd	3165.76a
$P_1M_1S_0$	0.30a	536.24a	29.20a	1263.78a	443.30cd	3547.44a
$P_1M_1S_1$	0.37a	725.99a	40.49a	2077.07a	608.27bc	4898.92a
$P_2M_0S_0$	0.30a	592.79a	31.07a	1498.37a	568.96bcd	3777.80a
$P_2M_0S_1$	0.38a	636.52a	34.08a	1788.15a	586.77bc	4114.24a
$P_2M_1S_0$	0.34a	707.79a	43.40a	2646.41a	554.40bcd	5243.16a
$P_2M_1S_1$	0.42a	599.26a	33.56a	1749.16a	908.77a	4069.00a

Means in a column followed by the same letter are not significantly different at $P\leq0.05$.

CONCLUSION

Overall, this experiment showed the significant effect of chemical P and *Mycorrhiza* on all the measured traits; however, *Pseudomonas* had a significant effect only on plant dry weight, the number of filled grains in plant, and P content.

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