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Effect of Arbuscular Mycorrhiza fungi application on distribution of phosphorus forms in rhizosphere soils of sunflower (*Helianthus annuus* L.)

Afshin Morovvat[1]*, Abdolmajid Ronaghi[1], Mehdi Zarei[1] , Mostafa Emadi[2], Mohammad Bagher Heidarianpour[1], Leila Gholami[1]

[1] Department of Soil Science, College of Agriculture, Shiraz University, Shiraz, Iran
[2] Department of Soil Science, Sari University of Agricultural Sciences and Natural Resources, Sari, Iran
*Corresponding author: amorovvat@inbox.ru

Appropriate management of soil phosphorus (P) fertility in highly calcareous soils of Iran as around the world should rely upon sound knowledge about the phosphorus reserve and its bioavailability. Despite numerous reports on the positive effects of vesicular arbuscular mycorrhizae (VAM) fungi on phosphorus uptake which is associated to Ectomycorrhiza as a branch of two major branches of group of fungis from mycorrhizal association, surprisingly little data exist on impact of VAM fungi on distribution of soil phosphorus forms in soils. A greenhouse-based study was conducted to investigate the potential effects of Arbuscular Mycorrhizal (AM) fungi application on phosphorus inorganic forms of soil rhizosphere in sunflower plants (*Helianthus annuus L.*). Results indicated that there is a significant increase in Fe-P fractions ($P<0.001$) in the rhizospher of the treated sunflowers with AM inoculums compared with untreated sunflowers. It could be potentially attributed to increases in secretion of specific–iron chelates such as hydroxamate siderophore from sunflower roots in +AM sunflowers treatments. **[**Morovvat **et al. Effect of Arbuscular Mycorrhiza fungi application on distribution of phosphorus forms in rhizosphere soils of sunflower (*Helianthus annuus* L.). International Journal of Agricultural Science, Research and Technology, 2012; 2(2):77-82].**
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1. Introduction

Phosphorus (P) is second only to nitrogen as an inorganic nutrient needed for both plants and microorganisms (Johri et al, 1999). Phosphorus is also important in plant bioenergetics. As a component of adenosine triphosphate (ATP), phosphorus is needed for the conversion of light energy to chemical energy during photosynthesis (Hopkins and Hüner, 2009). Of the major plant nutrients, world resources of P are the smallest and, thus, P should be used as efficiently as possible in order to conserve the resource base on a global scale and to maintain and increase, where necessary, agricultural productivity (Syers et al, 2008).

There are two main P sources in agricultural soils: the natural geochemical background and the fertilization (Renneson et al, 2010). In agricultural systems, P is often applied to the soil as a chemical fertilizer. Most of the P applied in the soils is not taken up by the crop, but it is retained in insoluble forms or fixed as mineral forms in the farms even as high as 90% or more. Soil P can exist in various inorganic (Pi) and organic forms (Po). Specific determination of Pi can be obtained by fractionation methods. Native phosphorus in soils is derived mainly from apatite. During its weathering and soil development, phosphorus is liberated and adsorbed by plants and recycled, incorporated into the organic mater of soils and sediments, or redeposited as either insoluble or slowly soluble mineral forms (Bakari et al, 2004; Stevenson and Cole, 1999). Only a small fraction of this total phosphorus is in a form that is readily available to plants. Approximately 90 percent of the soil phosphorus occurs in insoluble or soluble forms that easily turn to fixed forms.

Microbial activity plays a major role in P transformation and redistribution into different inorganic and organic forms (Stewart and Tiessen 1987; Barančíková et al. 2007). The beneficial effect of arbuscular mycorrhiza on plant growth is mainly attributed to higher P uptake by plants (Sakurai et al. 2001). The arbuscular mycorrhizal symbiosis is a mutualistic association formed between plants and a wide variety of fungi from the phylum Glomeromycota. The symbiosis is formed by the majority of the vascular flowering plants and is found in ecosystems of around the world. In general, the symbionts trade nutrients, and the arbuscular

mycorrhizal (AM) fungus obtains carbon from the plant while providing the plant with an additional supply of phosphorus (Smith and Read, 1997; Gianinazzi-Pearson et al, 2006). There are many researches that tried to explain the beneficially of mycorrhizal symbiosis in the term of increasing of availability of phosphorus for plants (e.g. Li et al, 2006) but there is no research that show which form of inorganic phosphorus in soil affected by mycorrhizal symbiosis in calcareous soils. Therefore, the current research is an attempt to describe the effect of arbuscular mycorrhizal application on distribution of phosphorus forms in the rhizosphere soils of planted sunflower in greenhouse.

2. Materials and methods

Preparation of mycorrhizal inocula

Arbuscular mycorrhizal fungi used in this study (*G. intraradices*) was grown in sand matrix for 6 months prior to the beginning of the experiment in multispore pot culture with maize (*Zea mays* L.) as host plants, under controlled green house conditions (12-13 h photoperiod range, 18-40 ºC temperature range, 16-70 % relative humidity range). Each pot of mycorrhizal treatments received 50 gram of sand matrix containing colonized root fragments, hyphae and spores. Pots from control treatments received the same weight of inoculum's sand autoclaved twice (121 ºC for 30 min).

Experimental design

The experiment was a factorial design using a 2×2×2 with three replications factorial in randomized complete block design with two P levels (15 and 30 mg. Kg^{-1}) were applied as mono calcium phosphate, two mycorrhizal fungi treatments (inoculated and non inoculated with *G. intraradices*), two cultivars of maize (Maxima and Zola).

Maize seeds were germinated in plastic pots with about 2500 g soil. Each pot received 5 seeds. Pots were randomized in the greenhouse. After sowing, seedlings were reduced. The plants were maintained in a controlled greenhouse condition (12-13 h photoperiod range, 18-40 ºC temperature range, 16-70 % relative humidity range), were irrigated daily. The soil used in this study was collected at a site located in central Iran, Chitgar area in Fars province; which had low content of micro/macro elements except potassium and calcium needed by maize. Plants growing in the soil supplemented with modified fertilizer solution based on soil analysis.

Mycorrhizal colonization

To assess AMF colonization, the fresh fine root sub-samples were cut into approximately 1 cm pieces, steadied in 10% KOH, washed with deionised water, embedded in 2% HCl and stained using Fushin acid according to method described by Kormanik and McGraw (1982). Stained root samples were examined microscopically to asses the percentage of mycorrhizal colonization using the grid-line intersect method (Giovannetti and Mosse, 1980).

Soil Analysis

Soil samples were oven dried at 40 ºC for 48 h and passed through a 2 mm sieve. The soil pH was measured using a 1:2.5 (w/v) soil water ratio (Houba et al. 1995). CEC was determined using the sodium saturation method (Rhoades, 1986) Organic carbon (OC) was determined by the Walkley and Black method (1934) as modified by Allison (1965). Total nitrogen (TN) was determined with the Kjeldahl method (McGill and Figueiredo, 1993), Available phosphorus (P) was measured by the Olsen method (Olsen et al., 1954). Particle size distribution was determined by sedimentation according to hydrometer method (Gee and Bauder, 1986). ECe was measured in soil extraction by the use of conductivity meter which have been described in details by Rhoades (1986). Available micro nutrients contain (Zn, Fe, Cu and Mn) extracted by DTPA method and determined using atomic absorption spectroscopy. The results of all previous methods presented in Table 1.

Jiang and Gu (1986) sequential fractionation method was used to determine inorganic P forms in soil which is summarized in Figure 1.

Table 1. Main characteristics of the studied soils

Texture	Loam
pH	7.9
ECe (ds/m)	0.5
OM (%)	1.2
TN (%)	0.06
CCE (%)	45.2
Olsen-P ($mg.kg^{-1}$)	5.4
DTPA extracTable Cu ($mg.kg^{-1}$)	1.06
DTPA extracTable Zn ($mg.kg^{-1}$)	0.71
DTPA extracTable Mn ($mg.kg^{-1}$)	4.3
DTPA extracTable Fe ($mg.kg^{-1}$)	6.49

Statistical analysis

Statistical analysis was performed using the SAS software program. The data were analyzed by one-way analysis of variance (ANOVA). To detect the statistical significance of differences ($p < 0.05$) between means, the Tukey test was performed. Principal Component Analysis (PCA) was performed using XLState software.

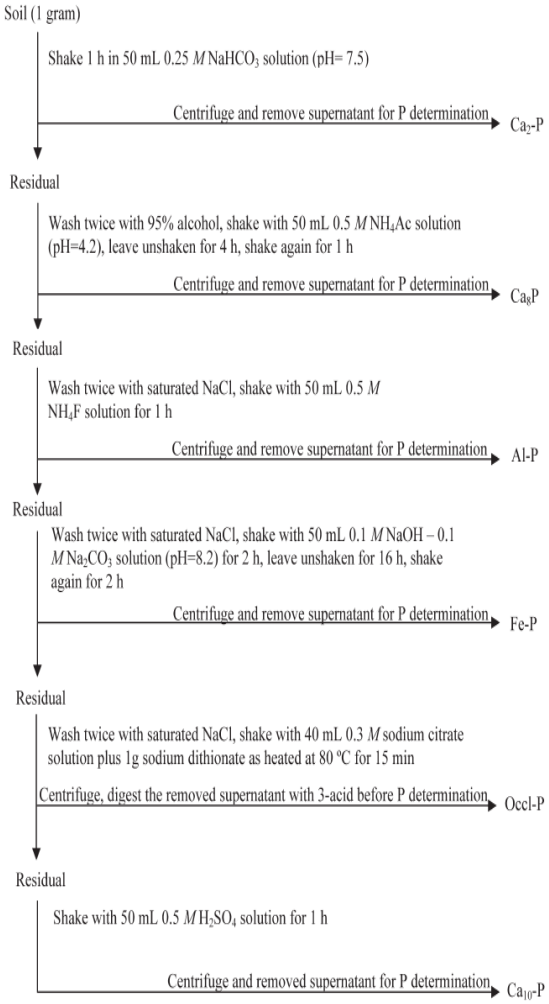

Figure 1. Sequential extraction procedure of soil inorganic P (Giang and Gu, 1986)

3. Results and discussion

In Table 2, effects of main treatments (mycorrhizal inoculums, primary added phosphorus levels and type of sunflower cultivars) and their interactions on soil phosphorus forms and mycorrhizal symbiosis percentage (MSP) at the end of vegetative stage were shown. The effects of all treatment on different soil P fractions are specified and discussed as following.

$NaHCO_3$ extractable P (Ca_2-P)

This fraction is assumed to resemble dicalcium phosphate. Results in Table 2 indicated that the primary added phosphorus levels and the type of cultivar have the direct impact on this fraction of phosphorus ($P<0.01$). However, Mycorhiza inoculation did not show any differences at significant level for Ca_2-P form. It could be potentially attributed to the high treated phosphorus to sunflowers that subsequently did prevent to emerge of fungi effects. Also there was no significant difference on types of sunflower cultivar in extraction of Ca_2-P. Tawaraya et al. (2006) stated that the AM colonization of plants inoculated with G. margarita and G. etunicatum was 86% and 54%, respectively. They also found that the adhesion of external hyphae was observed on the surface of the mullite ceramic tubes buried in soil of the hyphal compartment. They found that the colonization of AM fungi increased shoot P uptake and growth. Soil solution collected from the hyphal compartments of AM fungi solubilized more P than did that from uninoculated plants. It is suggested that hyphal exudates can contribute to increased P uptake of colonized plants.

NH_4AC extractable P (Ca_8-P)

This fraction is assumed to resemble octacalcium phosphate. Results in Table 3 revealed that a×b and a×c have significant ($P< 0.01$) impact on this fraction of phosphorus. Results showed that infected AS613 by AM fungi had more ability in increasing extraction of this fraction compared with other treatments. There is no significant difference between the primary added phosphorus levels in +AM treatments however in -AM treatments the ability of extraction were increased by increasing the added phosphorus level. Large secretion of $NH4^+$ component by AM fungi could be attributed for not changing the Ca_8-P in regard of changes in added P levels. $NH4^+$ decreased the pH in rizosphere soils and subsequently led to high extraction.

NH_4F extractable P (Al- P)

This fraction is assumed to resemble P adsorbed by Al oxides. Results in Table 4 showed that a×c and b×c interaction of the treatments have significant impact on this fraction ($P< 0.01$). As shown in Table 3, in –AM treatments the extraction ability on this P fraction with M5-54-1 was significantly higher than AS613 however there is no significant difference between cultivars in +AM treatments in amounts of extraction. More secretion of $NH4^+$ component from AS613 roots that complex the fluorine ion of extractant is the possible cause of more extraction of Al-P with M5-54-1 cultivar. The AM fungi apparently balanced the $NH4^+$ secretion and led to the decrease of pH.

Table 2. Amounts of Pr>F for each treatment and their interaction on soil P forms and MSP.

Main treatments	Sub-treatments	Means of Soil P forms						
		Ca2-P	Ca_8-P	Ca_{10}-P	Al-P	Fe-P	O-P	MSP[1]
Inoculationed AM	0	7.3678	58.423	108.869	20.223	4.766	94.8	0.3323
levels	1	7.9029	61.044	110.136	20.193	11.361	98.1	0.4859
LSD		1.2648	3.242	3.844	2.793	2.545	5.732	0.0459
Primary added P	0	5.2042	56.995	108.093	19.754	9.358	93.9	0.3822
levels	1	10.066	61.965	110.708	20.613	5.946	99	0.4428
LSD		1.2648	3.242	3.844	2.793	2.545	5.732	0.0462
Sunflower cultivars	M5-54-1	7.0202	57.794	108.073	20.576	6.566	98.1	0.4518
type	AS613	8.2505	61.266	111.02	19.882	9.047	94.8	0.3558
LSD		1.2648	3.242	3.844	2.793	2.545	5.732	0.0462
Source of variation				Pr>F[2]				
Mycorhizal inoculation(a)		0.1948	0.1642	0.2950	0.8287	<.0001	0.1919	0.0001
Primary added phosphorus(b)		0.0001	0.0101	0.1654	0.3775	0.0073	0.0509	0.0048
Sunflower cultivars(c)		0.0366	0.0413	0.1392	0.7032	0.0009	0.1797	0.0008
A×b		0.7618	0.0072	0.5327	0.0519	0.0048	0.0485	0.9464
A×c		0.4174	0.0087	0.8962	0.0264	0.0008	0.172	0.0008
B×c		0.2754	0.6674	0.9642	0.0288	0.7135	0.253	0.0419

[1] Mycorrhizal symbiosis percentage
[2] Decrease in the value of Pr>F showed the increase of significancy of the treatment effects.

Table3. Least square means for effect of AM×b and AM×c on Ca_8-P form (mg/kg), at Pr≤0.05.

AM	b* (ppm)	LSMeans	AM	c**	LSMeans
-	12	53.342b	-	M5-54-1	59.2b
-	27	63.502a	-	AS613	57.64b
+	12	60.7825a	+	M5-54-1	56.32b
+	27	60.4282a	+	AS613	64.88a

nflower cultivars *Primary added phosphorus

Table 4. Least square means for effect of AM×c and b×c on Al-P form (mg/kg), at Pr≤0.05.

c*	LSMeans	b** (ppm)	C	LSMeans
M5-54-1	22.36a	12	M5-54-1	18.38b
AS613	18.75a	12	AS613	21.12ab
M5-54-1	18.78a	27	M5-54-1	22.76a
AS613	21.59a	27	AS613	19.04ab

*Primary added phosphorus; ** Sunflower cultivars

Table 5. Least square means for effect of AM×b and AM×c on Fe-P form (mg/kg), at Pr≤0.05.

b* (ppm)	LSMeans	AM	c**	LSMeans
12	4.48c	-	M5-54-1	4.71c
27	4.83c	-	AS613	4.61c
12	17.93a	+	M5-54-1	7.43bc
27	9.13b	+	AS613	19.63a

Sunflower cultivars; * Primary added phosphorus

Table 6. Least square means for effects of AM × Primary added P levels on O-P form (mg/kg) at Pr≤

Primary added P levels (ppm) 12		Primary added P levels (ppm) 27	
AM+	AM-	AM+	AM-
93.16b	95.37b	104.82a	95.3b

Table 7. Least square means for effect of AM×c and b×c on MSP, at Pr≤0.05.

AM	C*	LSMeans	b** (ppm)	c	LSMeans
-	M5-54-1	0.34b	12	M5-54-1	0.44a
-	AS613	0.34b	12	AS613	0.29b
+	M5-54-1	0.56a	27	M5-54-1	0.46a
+	AS613	0.37b	27	AS613	0.41a

** Sunflower cultivars; * Primary added phosphorus

NaOH-Na_2CO_3 extractable P (Fe-P)

This fraction is assumed to resemble P adsorbed by Fe oxides. Results in Table 5 showed that a×b and a×c have the significant impact on this fraction of phosphorus. Least square means for a×b and a×c were statistically compared (Table 5). Results showed that there were no significant differences between added primary P levels in –AM treatments, but in +AM treatments the extraction ability in low level of P (12 ppm) significantly was higher than high level of P (27 ppm). Secretion of specific-iron chelates such as hydroxamate siderophore (Shaw et. al, 1990) from AM inoculated sunflower roots is the possible pathway for these observed results. These siderophores can potentially release the adsorbed P on iron oxides. Ion pair effect of added P accompanied with released P from siderophore could prevent the effect of P on the extraction ability of Fe-P fraction. Results also revealed that the secretion of the specific iron chelates released by the AS613 cultivar was higher than M5-54 cultivar.

Occluded-P (O-P)

This fraction is assumed to resemble P incorporated into Fe oxides. As shown in Table 6, a×b interaction of the treatments has the significant impact on this soil P fraction. Least square means for a×b interaction was statistically compared in Table 6. It could be apparently demonstrated that the increase in the extractability of O-P in combination of +AM treatment and high level of added P will be achieved.

H_2SO_4 extractable P (Ca_{10}-P)

This fraction is assumed to resemble P present as apatite. As shoen in Table 1, no treatments and their interaction has significant impact on this fraction in soils. This indicated that the primary P in soils could not be affected by the AM fungi and fertilization.

Mycorrhizal Symbiosis Percentage

The results indicate that a×c and b×c interactions have significant impact on colonization of mycorrhiza percentage. Least square means for a×c and b×c were statistically compared in Table 7. The results showed that mycorrhizal symbiosis percentage in M5-54-1 was more than AS613 in +AM treatments, but there was no significant difference between sunflower species in –AM treatments. With increasing the primary added P level on AS613 led to the increases in symbiosis percentage. Also P levels did not affect mycorrhizal colonization on M5-54-1 that high affinity of this cultivar to band with AM was suggested for this phenomenon. Ouahmane et al. (2007) found that a strong interaction between Khouribga rock phosphate amendment and fungus inoculation was detected for the leaf P content results. They also emphasized that the use of a mixture of native AM fungi combination may increase the chance of including one very effective fungal isolate, but also, create a more favorable environment for the development of ecosystems processes. Pi and organic P (such as polyphosphate) could be carried within the fungus by cytoplasmic streaming or by bulk flow to the plant root from external hyphae located in the soil (Schachtman et al., 1998).

4. Conclusion

Management of soil phosphorus fertility should rely upon sound knowledge of phosphorus reserves and bioavailability. A greenhouse-based study was conducted to investigate potential effects of AM application on soil rhizosphere (phosphorus inorganic forms) in sunflower plants. Results indicated that significant increase in Fe-P fractions ($P<0.005$) in the rhizospher of the treated sunflowers with AM compared with untreated sunflowers. This could potentially be attributed to the high secretion of iron-specific chelates such as hydroxamate siderophore from sunflower roots in +AM treatments. AM apparently induced high secretion of NH_4^+ components that led to no significant changes in the Ca_8-P and other P fractions in regard of increases in the primary added P levels.

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