Effect of VA-Mycorrhiza on Growth and Yield of Sunflower (*Helianthus annuus* L.) at Different Phosphorus Levels

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***Abstract*—**The effect of seed inoculation by VA- mycorrhiza and different levels of phosphorus fertilizer on growth and yield of sunflower (Azargol cultivar) was studied in experiment farm of Islamic Azad University, Karaj Branch during 2008 growing season. The experiment treatments were arranged in factorial based on a complete randomized block design with three replications. Four phosphorus fertilizer levels of 25%, 50% 75% and 100% P recommended with two levels of Mycorrhiza: with and without Mycorrhiza (control) were assigned in a factorial combination. Results showed that head diameter, number of seeds in head, seed yield and oil yield were significantly higher in inoculated plants than in non-inoculated plants. Head diameter, number of seeds in head, 1000 seeds weight, biological yield, seed yield and oil yield increased with increasing P level above 75% P recommended in non-inoculated plants, whereas no significant difference was observed between 75% and 100% P recommended. The positive effect of mycorrhizal inoculation decreased with increasing P levels due to decreased percent root colonization at higher P levels. According to the results of this experiment, application of mycorrhiza in present of 50% P recommended had an appropriate performance and could increase seed yield and oil production to an acceptable level, so it could be considered as a suitable substitute for chemical phosphorus fertilizer in organic agricultural systems.

***Keywords*—** phosphorus fertilizer, seed yield, sunflower, VA-mycorrhiza

I. Introduction

PHOSPHORUS is one the most essential elements for plant growth after nitrogen. However, the availability of this nutrient for plants is limited by different chemical reactions especially in arid and semi-arid soils [1]. Phosphorus plays a significant role in several physiological and biochemical plant activities like photosynthesis, transformation of sugar to starch, and transporting of the genetic traits. Sharma [2] reported that one of the advantages of feeding the plants with phosphorus is to create deeper and more abundant roots. Phosphorus causes early ripening in plants, decreasing grain moisture, improving crop quality and is the most sensitive nutrient to soil PH [3]. Malakooti and Nafisi [4] declared that the best PH for phosphorous uptake by plants is 6.5. Arpana et al. [5] reported that a great proportion of phosphorus in chemical fertilizer becomes unavailable to the plants after its application in the soil. They referred this to formation of strong bonds between phosphorous with calcium and magnesium in alkaline PH and the same bonds with iron and aluminum in acidic soils. The mobility of this element is very slow in the soil and can not respond to its rapid uptake by plants. This causes the creation and development of phosphorus depleted zones near the contact area of roots and soil in rhizosphere. Therefore, the plants need an assisting system which could extend beyond the depletion zones and help to absorb the phosphorus from a wider area by developing an extended network around root system [6].

The research on Mycorrhiza fungus and its role in soil and plant has been an interesting scientific subject since 1800. The presence of this fungus in rhizosphere provides with an advantageous and interactive symbiosis relationship between a higher plant root and a nonpathogenic fungus. Through receiving energetic carbon resources from plant, fungus facilitates the uptake of many inorganic nutrients such as phosphorus, zinc, molybdenum, copper and iron for it. The symbiotic relationship between Mycorrhiza and plants is one of the most abundant symbiotic activities in plant kingdom which exists in most of the ecosystems. Unfortunately, the neglectful interference of human activities such as over application of fungicides and frequent chemical phosphorous fertilizers application (mainly in intensive agricultural systems), have seriously threatened this advantageous symbiosis. Efforts to produce inoculants from Mycorrhiza fungi and to use it in proper environmental conditions, is a significant environmental friendly way to help plant growth and development through the enhancement of this natural phenomenon [1]. The significance of this practice, especially under low fertility conditions, has been very obvious. Photosynthesis improvement in plants through Mycorrhiza symbiosis is mainly due to the increase in transporting of inorganic elements from soil to plants.

One of the most important means to achieve the goals of sustainable agriculture is to extent the application of biological fertilizers. To reach this goal, it is necessary to moderate the use of chemical fertilizers and pesticides like through the time and in the mean time increase the soil organic matter content [6].

Sunflower, one of the potential oilseed crops for Iran has a high P requirement. Increased plant growth in the presence of mycorrhizal infection has been attributed mainly to the enhanced uptake of P. The beneficial effects of VAM

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inoculation on P uptake, growth and yield responses of sunflower in field experiment studies have not been carried out on sunflower. Hence, the effect of VAM on P availability, growth and yield of sunflower was investigated at different P level.

II. Materials and Methods

A. Inoculum Preparation of Arbuscular Mycorrhizal Species

The inoculum of arbuscular mycorrhizal species including *Glomus etunicatum*, *G. mosseae* and *G. intraradices* isolated from saline soils [7] were produced over a four-month period on sorghum plants under greenhouse conditions using sterilized sand [8, 9]. The nutrient requirements of plants were supplied. Plants were irrigated until flowering, and after two weeks plant shoots were harvested from the soil surface. The mixture of plant roots and sand were used as the inoculum. The inoculation potential of different isolates was tested using the MPN method [10, 11]. Sterilized soil concentrations of 0.1, 0.01 and 0.001 of AM were used for sorghum plantation, which were grown for 1 month. The plants were then harvested and the inoculation percentage on the sorghum roots was determined [11].

B. Experimental Design

The experiment was conducted in Research field of College of Agriculture, Islamic Azad University, Karaj Branch. The height of the experimental site from the sea level was 1312 m. The mean annual precipitation was 265.9 mm and the long term minimum and maximum monthly precipitation ranged species from 46.9 to 108.2 mm, respectively. The mean annual temperature was 13.5^0C while the mean maximum and minimum temperatures were 40 and -18^0C, respectively. The soil texture of the Research Site was sandy loam with a pH of 7.4. The soil chemical properties before the start of the experiment are presented in (Table I).

We used a factorial design with two factors: with and without AM inoculum, P fertilizer at four levels, P_1 (25% recommended), P_2 (50% recommended), P_3 (75% recommended) and P_4 (100% recommended), on the basis of a randomized complete block design in three replicas. Each plot consisted of 4 rows, 10 m long with 60 cm spaced between rows and 17 cm distance between plants on the rows. The sunflower (Azargol cultivar) was planted at 10 plants m^2 density. Seedbed preparation was done in early spring. Nitrogen fertilizer of 200 kg. ha^{-1} was used in the form of urea. Nitrogen fertilizer was top dressed in three portions, one third at the time of planting, one third in V_6 stage and the remain before flowering. To inoculate the seed by biological fertilizers, the seed were first covered by Arabic Gum solution and then Mycorrhiza was applied according to experimental treatments.

In the flowering stage, root colonization by AM was determined by preparing root samples at 0.5 g and one hundred root segments were examined for each sample. The stained roots were observed under a compound microscope. A root segment was considered as AM positive if it showed any fungal bodies like mycelium, vesicle and arbuscules. Percent of root colonization was calculated as follows:

$$\text{Root colonization (\%)} = \frac{\text{Number of AM positive segments}}{\text{Total number of segments observed}} \times 100$$

In the harvest stage, the two middle rows were used for sampling and measured parameters such as: plant height, number of seeds per head, 1000 seeds weight, seed yield, biological yield, harvest index, oil percentage and oil yield were assessed. Grain yield in each plot measured with 10% humidity.

Statistically of the result was done by using SAS [12]. Mean were compare using the Duncan's Multiple Range Test (DMRT) at 5% level of probability.

III. Results and Discussion

A. Root Mycorrhizal Colonization: A variety of studies suggest that phosphorous uptake by plant root can be enhanced when they are infected by arbuscular mycorrhiza (AM) fungi. In this study, Mycorrhizal colonization fluctuated from 2.7 in treatment (M_0P_3) to 36.3 in treatment (M_1P_1). The main effects as well as interaction effect were affected by mycorrhizal colonization. Thus, it can be concluded that the high rate of chemical phosphorus fertilizers application, lead to antagonistic interaction with mycorrhiza. in low amount of chemical phosphorus fertilizer, mycorrhiza was able to increase the root colonization significantly.

B. Plant Height: The analysis of variance (Table II) showed no significant effect of phosphorus fertilizer, Mycorrhiza treatment and their interaction effects on plant height. It seems that phosphorus does not play an important role in enhancement of plant height.

C. The number of seeds per head: the number of seeds per head was significantly affected by mycorrhiza and phosphorous levels, but no in their interaction effect. (Table II). Mycorrhizal plants had about 7% more number of seeds in head in compared of non-mycorrhizal plants. It means that mycorrhiza plays an important role in sunflower generative growth and therefore to make a significant increase in the number of seeds per head. Tohidi-Moghaddam et al. [13] reported that phosphorous solubilizing microorganisms increase the available phosphorous in the soil which could enhance the seed number in plant.

D.Seed Yield: Mycorrhiza and phosphorus fertilizer had significantly effects on seed yield, but interaction of theirs had no effect on the seed yield. Mycorrhiza could with symbiosis activity itself, cause to increasing P nutrient in around root plants and addition absorb by roots. At all the levels of phosphorous fertilizer, the mycorrhiza plants had higher seed yield. In fact, at 25% level of P fertilizer, the mycorrhizal plants had 13% higher seed yield compared to non-mycorrhizal plants, while at 50%, 75% and 100% P recommended, mycorrhizal association resulted in higher seed yield of 10%, 4% and 2%, respectively. The seed yield in M0P4 (4.735 t. ha^{-1}) had no significant effect with M1P2 (4.524 t. ha^{-1}). The results of using biological fertilizer treatment (inoculation by mycorrhiza) with 50% phosphorous recommended was not significantly different from the high rate of chemical phosphorous application (M0P4 treatment). According to the results of this experiment, application of mycorrhiza in present of 50% P recommended had an appropriate performance and could increase grain yield and oil production to an acceptable level, so it could be considered as a suitable substitute for chemical phosphorus fertilizer in organic agricultural systems.

E. Biological Yield: biological yield was significantly affected by phosphorus fertilizer (Table II) but mycorrhiza had no significant effect on this trait. The maximum biological yield of 12.616 t. ha^{-1} obtained in P4 (100% P recommended) which was not significantly different from P3 and P2 treatment, and the minimum biological yield of 11.024 t.ha^{-1} was obtained in P1 (25% P recommended). On the basis of this study, it seems that mycorrhiza and phosphorous fertilizer did not have much positive effects on biological yield. It can be also concluded that the initial soil phosphorous content already adequate without phosphorous fertilizer application.

F. Harvest Index: among the all of treatment, highest and lowest harvest index obtained in (M1P1) with average 38.9 % and (M0P2) with average 34.2 %, respectively. Mycorrhizal plants were more successful than non-mycorrhizal plants (but were not significant) in transport of assimilate from source to sink parts and produced high harvest index. One of benefit effects of mycorrhiza is on plants photosynthesis, VAM plants often display higher rate of photosynthesis than NM counterparts do, which is consistent with VAM effects on stomatal conductance. Most of the researchers suggested that VAM symbiosis increased the units of photosynthesis, and so as to increase the rate of photosynthetic storage and export at the same time [14].

G.Oil ***Yield:*** oil yield was significantly affected by mycorrhiza and phosphorous fertilizer levels (Table II). The maximum oil yield of 2.198 t.ha^{-1} was obtained in 100% P recommended which was not significantly different from 75% P recommended level, and the minimum yield of 1.794 t.ha^{-1} was obtained using 25% P recommended (Table III). Also, Mycorrhizal plants had about 10% more oil yield in compared of non-mycorrhizal plants. according to the positive effect of mycorrhiza on oil yield, it can be concluded that to increase oil yield in sunflower, use of biological fertilizers is preferred to the application of chemical ones. This can partially encourage farming with the mere use of biological fertilizers (organic systems).

IV. Conclusions

Results from the present study indicated that head diameter, seed number in head, seed yield and oil yield have been affect significantly by inoculation with Mycorrhiza, because this biofertilizer can enhance absorption of phosphorous by plant.

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TABLE I
SOIL PHYSICAL AND CHEMICAL PROPERTIES OF EXPERIMENTAL AREA

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Soil texture	PH	E.C (ds/m)	Organic Carbon (%)	Total N (%)	Available P (P. P. M)	Available K (P. P. M)
0 - 30	55.0	20.0	25.0	Sand loam	7.4	4.6	0.47	0.03	4.5	252
Optimum				loam	6.5 – 7.5	2.0<	>1.0	1.0>	10 - 15	200 - 300

TABLE II
ANALYSIS VARIANCE OF MEASURED PARAMETERS

S.O.V	d.f	Mycorrhizal Colonization	Plant height	Head diameter	Seed number in head	1000-seed weight	Seed Yield	Biological Yield	Harvest Index	Seed oil	Oil yield
Rep	2	4.041^{ns}	5.680^{ns}	3.110^{ns}	125.305^{ns}	30.616^{**}	0.257^{ns}	0.215^{ns}	8.271^{ns}	2.183^{ns}	0.066^{ns}
M	1	1998.375^{**}	10.269^{ns}	5.616^{*}	22669.327^{*}	0.338^{ns}	0.597^{*}	0.546^{ns}	18.361^{ns}	13.801^{ns}	0.247^{*}
P	3	191.152^{**}	9.354^{ns}	11.717^{**}	9433.758^{*}	16.864^{**}	0.624^{**}	2.950^{*}	4.860^{ns}	11.487^{ns}	0.239^{**}
M*P	3	162.708^{**}	3.961^{ns}	0.393^{ns}	2296.849^{ns}	2.794^{ns}	0.072^{ns}	0.026^{ns}	8.419^{ns}	0.069^{ns}	0.013^{ns}
Error	14	13.279	23.162	1.698	2839.242	2.167	0.093	0.788	16.877	7.551	0.038
C.V (%)		29.1	2.8	7.7	7.8.	2.7	6.9	7.4	11.1	6.2	9.9

*: Significant at 0.05 level, **: Significant at 0.01 level, ^{ns}: No significant difference.

TABLE III
MEAN COMPARISONS OF THE MAIN EFFECTS

Treatment	Mycorrhizal Colonization (%)	Plant height (cm)	Head diameter (cm)	Seed number in head	1000-seed weight (gr)	Seed Yield $(ton.\ ha^{-1})$	Biological Yield $(ton.\ ha^{-1})$	Harvest Index (%)	Seed oil (%)	Oil yield $(ton.\ ha^{-1})$
M levels										
M_0	3.4^{b}	170.7^{a}	14.4^{b}	908.9^{b}	54.3^{a}	4.287^{b}	11.855^{a}	36.2^{a}	43.5^{a}	1.871^{b}
M_1	21.7^{a}	172.1^{a}	15.3^{a}	964.2^{a}	54.1^{a}	4.603^{a}	12.156^{a}	38^{a}	45.1^{a}	2.074^{a}
P levels										
P_1 (25%)	20.2^{a}	169.8^{a}	13.2^{c}	901.1^{b}	52.1^{c}	4.066^{c}	11.024^{b}	35.9^{a}	42.8^{a}	1.749^{c}
P_2 (50%)	13.3^{b}	171.2^{a}	14.4^{bc}	910.5^{ab}	54.5^{ab}	4.300^{bc}	12.004^{ab}	36.9^{a}	43.4^{a}	1.875^{bc}
P_3 (75%)	9.2^{bc}	171.7^{a}	15.5^{ab}	968.5^{a}	54.2^{b}	4.634^{ab}	12.379^{a}	37.5^{a}	44.7^{a}	2.069^{ab}
P_4 (100%)	7.5^{c}	172.8^{a}	16.4^{a}	966^{a}	56.2^{a}	4.780^{a}	12.616^{a}	38^{a}	46^{a}	2.198^{a}

Means which have at least one common letter are not significantly different at the 5% level using (DMRT).

TABLE IV
MEAN COMPARISONS OF THE INTERACTION EFFECT

Treatment	Mycorrhizal Colonization (%)	Plant height (cm)	Head diameter (cm)	Seed number in head	1000-seed weight (gr)	Seed Yield $(ton.\ ha^{-1})$	Biological Yield $(ton.\ ha^{-1})$	Harvest Index (%)	Seed oil (%)	Oil yield $(ton.\ ha^{-1})$
M_0P_1	4.0^{d}	168.9^{a}	12.9^{c}	663.7^{b}	51.4^{c}	3.789^{c}	10.898^{b}	34.8^{a}	41.9^{a}	1.591^{c}
M_1P_1	36.3^{a}	170.8^{a}	13.4^{c}	738.6^{ab}	52.8^{bc}	4.343^{bc}	11.150^{ab}	38.9^{a}	43.8^{a}	1.906^{abc}
M_0P_2	3.7^{d}	171.1^{a}	13.9^{bc}	663.1^{b}	55.3^{ab}	4.077^{bc}	11.934^{ab}	34.2^{a}	42.7^{a}	1.752^{bc}
M_1P_2	23.0^{b}	171.2^{a}	14.9^{abc}	758^{a}	53.7^{bc}	4.524^{ab}	12.073^{ab}	37.6^{a}	44.1^{a}	1.999^{ab}
M_0P_3	2.7^{d}	170^{a}	14.6^{abc}	757.2^{a}	54^{abc}	4.548^{ab}	12.159^{ab}	37.4^{a}	43.9^{a}	1.995^{ab}
M_1P_3	15.7^{c}	173.4^{a}	16.3^{ab}	779.9a	54.4^{ab}	4.720^{a}	12.599^{ab}	37.5^{a}	45.4^{a}	2.142^{a}
M_0P_4	3.4^{d}	172.9^{a}	16.1^{ab}	751.6^{ab}	56.7^{a}	4.735^{a}	12.427^{ab}	38.4^{a}	45.3^{a}	2.146^{a}
M_1P_4	11.7^{c}	172.8^{a}	16.7^{a}	780.3^{a}	55.6^{ab}	4.825^{a}	12.804^{a}	37.7^{a}	46.7^{a}	2.251^{a}

Means which have at least one common letter are not significantly different at the 5% level using (DMRT)