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EFFECTS OF MYCORRHIZA, GYPSUM AND PHOSPHORUS APPLICATIONS ON WHEAT PLANT GROWTH AND NUTRIENT UPTAKE

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Abstract

A pot experiment was carried out to determine the effects of phosphorus as triple super phosphate and sulfur as $CaSO_4$ (gypsum) applications along with mycorrhiza inculcation on wheat plant growth and nutrient uptake. Gypsum was applied as equivalent to 0, 40 and 80 kg S ha^{-1} with 0, 40 and 80 kg of P_2O_5 ha^{-1}. Glomus mossea was used as an inoculant. The highest infection rate determined in 40 kg ha^{-1} S applied pots as 67%. Based on the mean values both S and P applications were decreased the infection rate. The highest shoot dry weight value was in mycorrhiza inoculated control plot which S and P were not applied (3.21 g); however, based on the mean values no statistical differences determined between mycorrhiza inoculated and non-inoculated pots. Mycorrhizal inoculation increased nitrogen contents of the plants, the highest mean nitrogen value gathered from mycorrhiza applied pots as 1.49%. Although the rather higher infection rate observed in mycorrhiza inoculated pot, P concentration was not influenced from these considerable infection rates. Ca concentration was significantly improved from P application, whereas Mg concentration was not affected from any of the treatment tested. Fe concentration of the plant was the highest in mycorrhiza inoculated and 80 kg ha^{-1} P_2O_5 applied pot as 45 mg kg^{-1}, while there was no influence of gypsum application on Fe. Similarly, Zn contents was influenced from P application but other treatments were not effective. Surprisingly mycorrhiza inoculation was not effective as expected, even some parameters were adversely effected.

Key words*: mycorrhiza, plant nutrition, gypsum, phosphorus fertilization.*

INTRODUCTION

Mycorrhiza has great influence on nutrient uptake of the plants that growing in calcareous soil where soil pH is generally high. As two third of the soils in Turkey have deficiencies of Fe, Zn or both (Eyupoglu et al., 1998), sulfur containing additives are widely recommended to overcome this nutritional disorders. On the other hand mycorrhizal inoculation is biological approach to improve plant micronutrient uptake which economic and environmentally friendly. The presence of indigenous mycorrhizal has a potential enhancement on the growth and on the root morphology under arid and semiarid conditions (Almaca and Ortas, 2010). Almaca et al. (2013a) reported 5.4% and 12.7% yield increase by mycorrhiza inoculation while *Glomus mossea* species reported more effective on pepper yield then *Glomus etunicatum*. Moreover, stimulating effects of phosphorus on mycorrhizal contribution was also reported Almaca et al. (2013a). Sulphur is one of the deficient nutrients in the soil especially in the high pH condition (Almaca et al., 2013b), thus, sulphur application is recommended in those soils. Almaca et al. (2013b) reported 3.3-17.1% yield increase by depending on the Sulphur application rates. Arshadullahet al. (2013) recommended 150 kg ha^{-1} gypsum dose for maximum benefit in saline-sodic soils. The soils used in this experiment has slightly higher pH; therefore, maximum gypsum dose selected around half of Arshadullahet al. (2013) recommendation. The aim of this research was to evaluate the effects of gypsum and phosphorus applications on wheat plant growth and nutrient uptake in mycorrhiza inoculated and non-inoculated conditions.

MATERIALS AND METHODS

Experiment was carried out as a pot experiment at 2012-2013 growing season, using the soils of Harran series which located Sanliurfa, Turkey.

The basic properties of experimental soil are presented in Table 1. Relatively undisturbed 2 kg of soils were placed to pots that have a *Glomus mossea* mycorrhiza spores containing culture layer which embed 5 cm deep from the soil surface. Firat 93 wheat seeds were used as a test plant.

Table 1. Basic properties of experimental soil

Texture	C
Organic matter (%)	1.3
EC (dS/m)	1.22
pH (1:2.5)	7.87
$CaCO_3$ (%)	24.6
P_2O_5 (kg da^{-1})	5.3
K_2O (kg da^{-1})	199

Each pot was fertilized by nitrogen as ammonium nitrate that equivalent to 100 kg N ha^{-1}. The experimental design was factorial which mycorrhizal inoculation (M+: inoculated or M-: non-inoculated), $CaSO_4$ and phosphorus incorporations were the factors. The doses of $CaSO_4$ were equivalent to 0 (S0), 40 (S40) and 80 (S80) kg ha^{-1} of Sulphur (S) whereas phosphorus were provided from triple super phosphate which equivalent to 0 (P0), 40 (P40) and 80 (P80) kg ha^{-1} of P_2O_5.

Analytical

The texture, pH (1:2.5), EC, $CaCO_3$, organic matter, available phosphorus, exchangeable potassium and magnesium properties of the experimental soil were evaluated by the methods described by Bouyoucous (1951), Jackson (1958), Richards (1954), Hizalan and Unal (1966), Walkey (1947), Olsen et al., 1954) and Thomas (1982).

When the plants were reached to desired harvest stage they have been cut near above soil surface, washed thoroughly and dried at 65 °C until they reach constant weight. All samples were grinded by agate mill (Walsh and Beaton, 1973). To analyze P, K, Fe, Cu, Zn and Mn, 0.2 g of samples were dry digested at 550 °C for 5 hour. The 2 ml of 1/3 HCl solution was added to each sample then filtered to 20 ml capacity container. Phosphor contents of samples were determined according to Murphy and Riley (1962) using spectrophotometer at 882 nm. The K, Zn, Fe, Cu, Mn, Ca and Mg concentration determined by atomic absorption spectrophotometer (Kacar, 1972). Total nitrogen contents were determined by Kjeldahl method (Bremner, 1965). Biomass developments were determined by analytical balance. Results obtained statistically analyzed by MSTAT-C software (Crop and Soil Sciences Department, Michigan State University, Version 1.2) according to randomized block design. Means were grouped using Duncan test at 0.05 alpha level (Efe et al., 2000).

Mycorrhizal infection determination

At the end of the experiment roots were sampled, cleaned and stained according to Koske and Gemma (1989). According to this method, once the roots have been thoroughly washed and the dead roots have been removed. The remaining roots were placed into a petri dish and cut into lengths of 1 cm. Samples were transferred to test tubes (1 cm in diameter and 20 cm length), 2.5% (w:v) KOH solution were added. After 45 minute of the duration in 90 °C of water bath, KOH solution was discarded and 1% of HCl solution added to as much as overlaying the samples. Once the previously added acid has been removed from the tubes, sufficient amount of acidified Glycerol and Trypan Blue solutions were added to the same tubes. The constituent of the tubes once again transferred to clean petri dishes, 10 of tiny root pieces selected and they were placed to microscope slides. Afterwards, infection rates were determined according to Giovanetti and Mosse (1980) with a magnification of 40-60 under the microscope.

RESULTS AND DISCUSSIONS

The mycorrhizal infection rates which were influenced from mycorrhizal inoculation as well as gypsum ($CaSO_4$) and phosphorus applications are presented in Table 2. Mycorrhizal infections were positively affected by mycorrhiza spore inoculations; therefore the higher values obtained from mycorrhiza inoculated plants. Although the highest infection rate was in S40 variant as 67%, based on the mean values both S and P applications were decreased the infection rate.

Table 2. Infection rates

	S doses	P doses 0	P doses 40	P doses 80	Averages
M+	0	47 ab	63 ab	47 ab	52 a
	40	67 a	27 bc	20 bc	38 b
	80	30 bc	43 ab	17 bc	30 b
	Avg.	48 **a**	44 **ab**	28 **b**	40 **A**
M-	0	7 c	3 c	7 c	6 c
	40	7 c	7 c	3 c	6 c
	80	17 bc	17 bc	0 c	11 c
	Avg.	10 **c**	9 **c**	3 **c**	7 **B**
Averages		29 ***A***	27 ***AB***	16 ***B***	

No infection was observed in the highest dose of S and P applied pots indicating both applications have diminishing effects on mycorrhiza spores. Biomass weight (Table 3) was not in accordance with infection rates, where no statistical differences determined between mycorrhiza inoculated and non-inoculated pots. The highest values were in mycorrhiza inoculated S0P0 pot as 3.21 g. P doses were also not effective on plant dry weights. When mycorrhiza inoculation and P doses were evaluated together, it was found that the lower P application (P40) cause the highest dry biomass weight in inoculated pots, while, the highest P dose required to reach same amount of biomass in non-inoculated conditions.

Table 3. Biomass weight (g)

	S doses	P doses 0	P doses 40	P doses 80	Average
M+	0	3.21 a	3.12 ab	2.73 bc	3.02 a
	40	2.76 bc	3.04 ab	2.74 bc	2.85 a
	80	2.69 bc	2.97 ab	2.79 a-c	2.82 a
	Avg.	2.89 **ab**	3.04 **a**	2.75 **b**	2.89 **A**
M-	0	2.84 a-c	2.85 a-c	3.06 ab	2.92 a
	40	2.74 bc	2.53 c	3.07 ab	2.78 a
	80	2.94 a-c	2.83 a-c	2.86 a-c	2.87 a
	Avg.	2.84 **ab**	2.74 **b**	3.00 **a**	2.86 **A**
Average		2.86 ***A***	2.89 ***A***	2.88 ***A***	

Almaca and Ortas (2013a) reported increased pepper yield by increasing P addition; however, contribution of mycorrhizae on yield were higher under high level of P addition. In this results presented indicating that higher dose is necessary only in case of non-inoculated conditions.

Nitrogen contents of the shoot are presented in Table 4. As well known, mineral nitrogen in soil is rather dynamic which plants don't need any mediator to reach; however, in this particular case, mycorrhizal inoculation increased nitrogen contents of the plants, where the highest mean nitrogen value gathered from mycorrhiza applied pots. Unexpectedly, the higher N contents were achieved in both P0 and P80 deses, while P40 cause reduction of wheat N contents. When mean values of S and mycorrhizal inoculation interactions viewed together, inoculated pots provide higher N contents and increasing S dose reduced nitrogen contents ($p<0.05$). No effects were seen in M- and S dose interaction.

Table 4. Nitrogen contents (%)

	S Doses	P doses 0	P doses 40	P doses 80	Average
M+	0	1.70 a	1.48 a-d	1.59 a-c	1.59 a
	40	1.38 b-d	1.44 a-d	1.51 a-d	1.44 ab
	80	1.64 ab	1.29 cd	1.42 a-d	1.45 ab
	Avg.	1.57 **a**	1.40 **bc**	1.51 **ab**	1.49 **A**
M-	0	1.32 cd	1.27 d	1.44 a-d	1.34 b
	40	1.42 a-d	1.25 d	1.32 c-d	1.33 b
	80	1.28 d	1.35 b-d	1.48 a-d	1.37 b
	Avg.	1.34 **c**	1.29 **c**	1.41 **bc**	1.35 **B**
Average		1.46 ***A***	1.35 ***B***	1.46 ***A***	

The values related the P contents of wheat plant are presented in Table 5. Only the P concentrations as a main factor were influenced from P doses. The P40 dose was not caused any changes on P concentrations whereas the P80 was provided the higher mean value as 0.30%. Although the quite higher infection rate observed in M+ (Table 2), P concentration was not influenced from these higher infection rates. This situation seems to be arising due to limited resources in the pots.

Table 5. Phosphorus contents (%)

	S doses	P doses 0	P doses 40	P doses 80	Average
M+	0	0.26 a-c	0.24 a-c	0.35 a	0.28 a
	40	0.24 a-c	0.21 c	0.27 a-c	0.24 a
	80	0.25 a-c	0.23 c	0.34 ab	0.28 a
	Avg.	0.25 **b**	0.23 **b**	0.32 **a**	0.27 **A**
M-	0	0.24 a-c	0.24 a-c	0.30 a-c	0.26 a
	40	0.25 a-c	0.29 a-c	0.26 a-c	0.27 a
	80	0.21 c	0.25 a-c	0.29 a-c	0.25 a
	Avg.	0.23 **b**	0.26 **b**	0.28 **ab**	0.26 **A**
Average		0.24 ***B***	0.24 ***B***	0.30 ***A***	

None of the factors such as mycorrhizal inoculation, gypsum and phosphorus applications were solely affected on K uptake (Table 6). But mycorrhiza-P interaction was significantly changed K concentration which the highest mean K values achieved in M+ and P0 (1.93%), and M- and P80 (1.75%) combinations. As clearly seen in Table 6, the effectiveness of mycorrhiza is reduced by increasing dose of P, while P application improved K concentration in M- condition.

Table 6. Potassium contents (%)

	S doses	P doses 0	P doses 40	P doses 80	Average
M+	0	1.92 a-c	1.63 b-d	1.77 a-d	1.77 a
	40	1.81 a-d	2.10 a	1.62 b-d	1.84 a
	80	2.06 ab	1.61 b-d	1.76 a-d	1.81 a
	Avg.	1.93 **a**	1.78 **ab**	1.71 **ab**	1.81 **A**
M-	0	1.74 a-d	1.65 b-d	1.67 a-d	1.69 a
	40	1.69 a-d	1.51 cd	1.81 a-d	1.67 a
	80	1.45 d	1.76 a-d	1.77 a-d	1.66 a
	Avg.	1.63 **b**	1.64 **b**	1.75 **ab**	1.67 **A**
Average		1.78 ***A***	1.71 ***A***	1.73 ***A***	

Phosphorus applications increased Ca concentration of the plant; however, neither mycorrhiza inoculation nor gypsum doses was effective on Ca (Table 7). In general, mycorrhiza-P dose interaction was stimulated Ca uptake where the highest values were determined from M+ and P80 dose.

Table 7. Calcium contents (%)

	S doses	P doses 0	P doses 40	P doses 80	Average
M+	0	0.55 ab	0.55 ab	0.62 a	0.57 a
	40	0.41 a-c	0.51 a-c	0.61 a	0.51 a
	80	0.47 a-c	0.39 a-c	0.65 a	0.50 a
	Avg.	0.48 **b**	0.48 **b**	0.62 **a**	0.53 **A**
M-	0	0.28 c	0.45 a-c	0.52 a-c	0.42 a
	40	0.51 a-c	0.34 b-c	0.51 a-c	0.45 a
	80	0.43 a-c	0.62 a	0.40 a-c	0.49 a
	Avg.	0.41 **b**	0.47 **b**	0.48 **b**	0.45 **A**
Average		0.44 ***B***	0.48 ***AB***	0.55 ***A***	

Magnesium concentration of the wheat plant was not influenced from any of the application tested in this research (Table 8). Mycorrhiza inoculation increased Mg uptake slightly which there is no statistical differences between M+ and M- ($p>0.05$).

Table 8. Magnesium contents (%)

	S doses	P doses 0	P doses 40	P doses 80	Average
M+	0	0.46 a	0.43 a	0.56 a	0.48 a
	40	0.38 a	0.55 a	0.64 a	0.52 a
	80	0.43 a	0.54 a	0.54 a	0.50 a
	Avg.	0.42 **a**	0.50 **a**	0.58 **a**	0.50 **A**
M-	0	0.29 a	0.45 a	0.42 a	0.39 a
	40	0.42 a	0.35 a	0.42 a	0.40 a
	80	0.52 a	0.56 a	0.34 a	0.48 a
	Avg.	0.41 **a**	0.46 **a**	0.40 **a**	0.42 **A**
Average		0.42 ***A***	0.48 ***A***	0.49 ***A***	

None of the application was significantly effective on Cu contents of the plant (Table 9). However, both gypsum and phosphorus reduced Cu uptake. Due to the fungicide features, cupper is one of the most widely used elements in both conventional and organic farming. Thus copper accumulation has recently been mentioned in the soils in Turkey, thus, gypsum and phosphorus applications would be recommended Cu accumulated soil to prevent excessive Cu uptake of the plants.

Table 9. Cupper contents (mg Cu kg^{-1})

	S doses	P doses 0	40	80	Average
M+	0	25 a	19 a	15 a	20 a
	40	18 a	17 a	13 a	16 a
	80	14 a	24 a	19 a	19 a
	Avg.	19 **a**	20 **a**	16 **a**	18 **A**
M-	0	10 a	13 a	8 a	10 a
	40	11 a	21 a	10 a	14 a
	80	19 a	10 a	13 a	14 a
	Avg.	13 **a**	14 **a**	10 **a**	13 **A**
Average		16 ***A***	17 ***A***	13 ***A***	

Fe and Zn deficiencies are very common in Turkey soil (Eyupoglu et al., 1998); however, none of the application was improved Fe concentration of wheat plant (Table 10). Only interactions of three of the applications significant on Fe contents, the highest value was observed in M+ S0 P80 interaction.

Table 10. Iron contents (mg Fe kg^{-1})

	S doses	P doses 0	40	80	Average
M+	0	32 a-c	36 a-c	43 a	37 a
	40	32 a-c	33 a-c	33 a-c	33 a
	80	35 a-c	29 bc	30 a-c	32 a
	Avg.	33 **a**	33 **a**	35 **a**	34 **A**
M-	0	29 c	32 a-c	34 a-c	31 a
	40	34 a-c	27 c	34 a-c	32 a
	80	38 a-c	41 ab	33 a-c	38 a
	Avg.	34 **a**	33 **a**	34 **a**	34 **A**
Average		33 ***A***	33 ***A***	35 ***A***	

Mycorrhizal infection encouraged Mn concentration where the higher mean Mn observed in M+. P doses were also improved Mn uptake, P80 provided the highest Mn content (Table 11).

Mycorrhizal inoculation did not significantly change the zinc content of the plant (Table 12). While gypsum doses did not affect Zn contents, increasing P doses increased Zn content and highest determined Zn content was in P80 dose.

Table 11. Manganese contents (mg Mn kg^{-1})

	S doses	P doses 0	40	80	Average
M+	0	38 a-c	31 b-e	45 a	38 a
	40	27 e	28 de	40 ab	32 b
	80	37 a-e	31 c-e	33 b-e	34 ab
	Avg.	34 **b**	30 **b**	39 **a**	34 **A**
M-	0	29 c-e	31 b-e	34 b-e	31 b
	40	36 a-e	29 c-e	32 b-e	32 b
	80	27 de	37 a-d	30 c-e	31 b
	Avg.	31 **b**	32 **b**	32 **b**	32 **B**
Average		32 ***AB***	31 ***B***	36 ***A***	

Table 12. Zinc contents (mg Zn kg^{-1})

	S doses	P doses 0	40	80	Average
M+	0	19 bc	19 bc	24 a-c	21 a
	40	19 bc	23 a-c	21 a-c	21 a
	80	17 c	25 a-c	31 a	24 a
	Avg.	18 **c**	22 **a-c**	25 **a**	22 **A**
M-	0	17 bc	18 bc	27 ab	21 a
	40	19 bc	17 bc	22 a-c	19 a
	80	20 bc	24 a-c	25 a-c	23 a
	Avg.	19 **bc**	20 **a-c**	24 **ab**	21 **A**
Average		19 ***B***	21 ***B***	25 ***A***	

CONCLUSIONS

The results gathered in this experiment were slightly out of expectations where mycorrhizal inoculation did not influence concentration of several plant nutrients analyzed. Infection rate values clearly indicate successful mycorrhizal inoculation; however, biomass weight, P, K, Ca, Mg, Cu, Zn and Fe concentrations were not influenced from mycorrhizal inoculation. P application was reduced infection rate in inoculated variants while improved P, Ca, Mn and Zn concentrations. Gypsum was also reduced infection rate; however, increased nitrogen concentration in mycorrhiza applied pots.

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