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THE EFFECTS OF DIFFERENT ZN DOSES AND MYCORRHIZAE APPLICATION ON HORSE BEAN GROWTH AND NUTRIENT UPTAKE UNDER STERILE AND NON STERILE SOIL CONDITIONS

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Abstract

Due to semi-arid Mediterranean climate conditions and soil properties, south east Turkey's soils usually do not contain sufficient amount of plant nutritious elements, especially phosphorous (P) and zinc (Zn). This scarcity conditions is one of the factors limiting agriculture productivity and fertility and consequently, decrease the crop production. Mycorrhiza can be able to increase soil fertility and enhance nutrient uptake and plant growth. The aim of this study was to investigate the effects of mycorrhizal inoculation and different Zn doses on horse bean (Vicia faba L.) growth and nutrient uptake under sterile and non-sterile soils conditions. The experiment was carried out under greenhouse conditions with three replications. Horse bean was used as a test plant and Funneliformis mosseae was used as mycorrhiza species (1000 spore plant^{-1}). Sterile and non-sterile Karaburun soils series from Çukurova region were used. Two doses of Zn (0 and 5 mg kg^{-1}) were used as fertilizer.
In both sterilized, non-sterilized soils mycorrhizal inoculation increased shoot dry matter and nutrient concentration. The results shown that shoot dry matter and nutrient concentration significantly increased of horse bean plants in non-sterilized soils. Maximum value of % P and Zn concentration was determined and in non-sterilized soils mycorrhiza inoculated have 0.28 % P (0 mg kg^{-1}Zn) and in mycorrhiza inoculated sterile soils, 52.1 mg kg^{-1}Zn (5 mg kg^{-1}Zn). The results are encouraging that the mycorrhiza can be used as an organic fertilizer for plant production under large arable conditions.

Key words*: mycorrhizae, horse bean, zinc, phosphorus.*

INTRODUCTION

Middle East-originated horse bean (*Vicia faba* L.) is among the earliest cultured legumes after chickpea and pea. Horse bean is commonly grown for human nutrition, but it is also used as animal feed (Prolea, 2014). Turkey, especially the Mediterranean Region, has loamy soils with high clay and low organic matter contents because of climate zone and geographical location. Undesired physical, chemical and biological soil characteristics also reduce available plant nutrient concentrations. Soils of Çukurova Region (Eastern Mediterranean Region) with intensive agricultural practices experience nutrient deficiency place to place mostly based on soil parent material and especially phosphorus (P) and zinc (Zn) deficiencies are the common cases in these soils (Ibrikci, 1994).

Mycorrhizal symbiosis between plants and fungi has been one of the most well-known plant-fungus associations since about 450 million years and is of significant importance for growth and persistence of terrestrial plants (Redecker et al., 2000). Obligate symbiotic arbuscular mycorrhizal fungi (AMF) belongs to endomycorrhiza group of *Glomeromycota* family and the fungi is in symbiotic association with about 80% of the plant species (Smith and Read, 2008). Horse bean is also among these plant species which is significantly mycorrhizal dependent (Ortas, 2012a). Turkey has also grown horse bean which can form associations with mycorrhizal fungi (Kaschuk et al., 2010). Plant roots are not the only dominant factors in plant nutrient uptake, but there are some other factors effective in nutrient uptake processes of the plants. It was reported in previous studies that hyphae-producing fungi species, also called as mycorrhiza, which can be seen under microscope and have symbiotic associations with plant roots, may also provide significant supports in plant nutrient uptake from the soils (Ryan et al., 2008). Arbuscular mycorrhiza fungi (AMF) aid in enlargement of root surface area in rhizosphere area and alter rhizosphere pH, thus increase P uptake of plants from the soils (Ortas and Rowell, 2004; Ortas and Akpinar, 2011). In case of an efficient infection between the mycorrhiza and the plants, plants give 5-10% of the carbon to mycorrhiza fungi and in return mycorrhiza facilitates plant nutrient flow (Hodgeand and Storer, 2015) and water uptake (Augé, 2001). It has been found that mycorrhizal inoculation increased plant especially P, Zn and Cu concentrations when effective root infection occurs (Smith and Read, 2008). Besides plant growth, AMF has several positive impacts on soil quality (Celik et al., 2011).

Zn uptake mechanism of mycorrhiza is similar with the phosphorus uptake mechanism and supplies about 60% of the Zn provided to plants through mycorrhizal hyphae from outside the root region (rhizosphere) (Ortas et al., 2011). Arbuscular mycorrhiza not only facilities P uptake of the host plant, but also significantly increase Zn uptake of the plants (Ortas and Akpinar, 2011; Ortas, 2012b). Therefore, existence of mycorrhiza fungi hyphae is a quite significant issue for the uptake of slowly available plant nutrients like Zn. According to Thompson (1990), non-existence of Zn deficiency of the plant species grown in soils with low DTPA-extractable Zn concentrations mostly resulted from Zn supplied from the soils through AM.

Zn is a quite significant microelement for human health. Zn deficiency is experienced about 60% of Turkish soils (Eyüpoğlu et al., 1996), therefore mycorrhiza utilization in agricultural soils is a significant issue to prevent Zn deficiency. Mycorrhiza utilization may have a strategic importance especially in soils of inner semi-arid regions. This study was conducted to investigate the effects of different Zn doses and mycorrhiza inoculations on nutrient uptake and plant growth of horse beans grown in sterilized and unsterilized soils poor in plant nutrients.

MATERIALS AND METHODS

The experiment was conducted on Karaburun soil series (*Typic Xerorthent*) which is nutrient deficient (Table 1). Soil used in the investigation which is silt clay loam, slightly alkaline (pH 7.4), calcareous (23.2%), phosphorus content low (26.0 kg·ha^{-1}), and potassium content is sufficient (630 kg·ha^{-1}). The half of the soil was sterilized by using an autoclave at 120°C for two hours. Two levels of Zn, 0 and 5 mg·kg^{-1} soil, as zinc sulfate ($ZnSO_4.7H_2O$) were applied.

Plants were inoculated with the AMF *Funneliformis mosseae*. A level of 1000-spore per pot was placed 3 cm below the seeds. The non-inoculated pots received the same amount of mycorrhizal spore-free inoculum.

Horse bean (*Vicia faba* L.) was used as a test plant. In the study, plastic pots with a capacity of 3 kg were used and 5 seeds were planted at the beginning of each pot. After germination, they were reduced to 3 plants. Distilled water was added daily to maintain the moisture at 75% of field capacity.

At the harvest of each pot, total plant biomass (dry weight of root and shoot) were recorded. Plant materials, shoots and roots were washed thoroughly with distilled. Then plants were oven-dried at 65°C for 48 h. The dry material was ground using a Tema mill, and 0.2 g of the ground plant material was ashed at 550°C, then dissolved in 3.3% HCl. The concentration of phosphorus was determined according to Murphy and Riley (1962). An ICP-spectrophotometer (ICP-OES; Thermo ICAP7000) was employed to determine the concentration of K, Zn and Fe in the plant samples. The root staining procedure described by Koske and Gemma (1989). The percentage of AMF colonization was identified by method of Giovanetti and Mosse (1980).

Table 1. Some physical and chemical properties of Karaburun series soils used in experiments

Soil Depth	P_2O_5	K_2O	Fe	Zn	Cu	Mn	pH	$CaCO_3$	Texture
	$kg \cdot ha^{-1}$		$mg \cdot kg^{-1}$					%	
(0-30)	26.0	630.0	3.02	0.38	0.57	3.21	7.41	23.2	SiCL

Mycorrhizal Dependency (MD)

After harvest, the total dry weight of the seedlings was recorded and inoculation effectiveness of the seedlings by AMF was calculated using the following formula by Ortas (2012a):

$$(MD) = \frac{TDW(+M) - TDW(-M)}{TDW(+M)} \times 100$$

TDW= total dry weight; +M, inoculated plant; -M, non-inoculated plant

Statistical Analysis

All statistical analyses (Tukey test and correlation) were performed using the SPSS 22.0 for Windows computer program.

RESULTS AND DISCUSSIONS

Effects of different Zn doses and mycorrhiza inoculation on shoot (SDW) and root dry weight (RDW) production of horse bean were investigated and results are presented in Table 2. Present findings revealed that mycorrhiza inoculations significantly increased both shoot and root dry matter production. While shoot dry matter production was 7.62 g pot^{-1} and 7.44 g pot^{-1} in unsterilized and sterilized control treatment respectively (Table 2). With 5 mg kg^{-1}Zn and mycorrhiza treatments, SDW was 8.36 g pot^{-1} under sterilized and 8.08 g pot^{-1} under the sterilized soil conditions. The greatest shoot dry matter production was observed as 9.18 g pot^{-1} in mycorrhiza-inoculated and non-Zn-treated sterilized control soil. Similar findings were also observed in root dry matter productions. Mycorrhiza had positive impacts on plant growth and development under both sterilized and unsterilized soil conditions. Considering the general averages, it was observed that the greatest shoot dry matter production of horse bean plants in unsterilized soils was 8.21 g pot^{-1}, the greatest root dry matter production was observed as 7.63 g pot^{-1} in sterilized soils. Similarly, Akpinar (2011) also reported that AM inoculations significantly increased dry matter production of horse bean plants. Also Ortas (2012a) showed that during 1997 to 1999 mycorrhizal inoculation significantly increased the horse bean yield under field conditions. Following the soil sterilization, since the other microorganisms, existing in soil and competing with plant roots for nutrients, were removed from the soil, mycorrhiza fungi had much more impacts on plant growth and development. Indigenous mycorrhiza also exists in unsterilized soils to some extend and thus had positive impacts on plant growth and development also in unsterilized soils (Ortas, 2003).

Table 2. Effects of different zinc doses and mycorrhiza inoculation on shoot and root dry matter production of horse bean plants grown in sterilized and unsterilized soils

			Shoot Dry Weight	Root Dry Weight
			g pot^{-1}	
(-) Sterile	**(–M)**	**(0 mg kg^{-1}Zn)**	7.62 $\pm 0.5^{b}$	6.94 $\pm 0.0^{ab}$
		(5 mg kg^{-1}Zn)	7.68 $\pm 0.1^{ab}$	5.71 $\pm 0.6^{b}$
	(+M)	**(0 mg kg^{-1}Zn)**	9.18 $\pm 0.9^{a}$	7.61 $\pm 0.2^{ab}$
		(5 mg kg^{-1}Zn)	8.36 $\pm 0.0^{ab}$	9.56 $\pm 1.3^{ab}$
		General Average	8.21^{A}	7.45^{A}
(+) Sterile	**(–M)**	**(0 mg kg^{-1}Zn)**	7.44 $\pm 0.2^{b}$	7.36 $\pm 0.1^{ab}$
		(5 mg kg^{-1}Zn)	7.81 $\pm 0.4^{ab}$	6.27 $\pm 0.1^{ab}$
	(+M)	**(0 mg kg^{-1}Zn)**	8.69 $\pm 0.0^{ab}$	9.88 $\pm 2.5^{ab}$
		(5 mg kg^{-1}Zn)	8.08 $\pm 0.1^{ab}$	7.02 $\pm 0.1^{a}$
		General Average	8.00^{B}	7.63^{A}

± Standard error, $P<0.05$

The greatest root infection was observed as 65% in 5 mg kg^{-1} Zn-treated and mycorrhiza-inoculated sterilized soils (Table 3). The reason of that high infection rate was considered as absence of other organisms to compete with mycorrhiza. The mycorrhizal inoculum increased the root colonization of horse bean plants compared with the non-inoculated treatments (Ortas, 2012a). Similar with the present findings, previous researchers also reported that Zn fertilization did not influence mycorrhiza infections (Ortas et al., 2002; Subramanian et al., 2009).

Mycorrhizae dependency (MD) was calculated after harvest. As can be seen in the table, MD increased with non-sterile soil. At non sterile soil was 19.31 %, and sterile soil was 13.50 % as calculated (Table 3). This means that mycorrhiza inoculation at the sterile soil was not sufficient. Also, mycorrhizal dependency was found higher in Zn applications as 25.29 %. Ortas (2012a) found that horse bean showed the highest mycorrhizal dependency in all 3 years, especially in P0 plots under field conditions. Tawaraya (2003) reported that mean MD values of 44% for field crops (37 species).

When the present findings were assessed for root infection and mycorrhizal dependence, root infections were observed in control treatments of sterilized soils even at quite low levels because of the existence of indigenous mycorrhiza. Since non-sterile soils have indigenous mycorrhizae which may significantly increase plant growth. As can be seen in Table 2, there is small differences in dry weight in between sterile and non-sterile soil treatments.

Table 3. Effects of different zinc doses and mycorrhiza inoculation on root infection and mycorrhizal dependence of horse bean plants grown in sterilized and unsterilized soils

			Root Infection %	Mycorrhizal Dependency %
		(0 mg kg^{-1}Zn)	10 ±0,0[c]	
(-) Sterile	**(–M)**	**(5 mg kg^{-1}Zn)**	5 ±7.1[c]	
		(0 mg kg^{-1}Zn)	15 ±7.1[c]	13.33
	(+M)	**(5 mg kg^{-1}Zn)**	15 ±7.1[c]	25.29
		General Average	11.3[B]	19.31
		(0 mg kg^{-1}Zn)	0 ±0.0[c]	
(+) Sterile	**(–M)**	**(5 mg kg^{-1}Zn)**	0 ±0.0[c]	
		(0 mg kg^{-1}Zn)	40 ±0.0[b]	20.30
	(+M)	**(5 mg kg^{-1}Zn)**	65 ±7.1[a]	6.71
		General Average	26.3[A]	13.50

± Standard error, $P<0.05$

When the present findings were assessed for plant nutrients, it was observed that while the lowest P concentration was 0.13% in 5 mg kg^{-1} Zn-treated and mycorrhiza-inoculated sterile soils and the greatest P concentration was 0.28% in mycorrhiza-inoculated unsterilized soils (Table 4). Although the differences between sterilized and unsterilized soils were not found to be significant, P concentrations generally increased with mycorrhiza inoculations as compared to control treatments. It was reported that mycorrhiza fungi significantly increased the uptake of slowly available nutrients, especially the phosphorus under controlled conditions (Grant et al., 2005; Ortas and Akpinar, 2006; Ortas, 2012b). Similar with P concentrations, plant tissue potassium (K) concentrations also increased with mycorrhiza inoculations. With regard to Fe concentrations, the greatest value was observed as 308.4 mg kg^{-1} in 5 mg kg^{-1}Zn-treated and mycorrhiza-inoculated unsterilized soils. The lowest Zn concentration (26.7 mg kg^{-1}Zn) was observed in sterilized and non-mycorrhiza-inoculated control treatments and the greatest Zn concentration (52.1 mg kg^{-1}Zn) was observed in 5 mg kg^{-1}Zn-treated and mycorrhiza-inoculated sterilized soils. Previous researchers also indicated that mycorrhiza treatments increased uptake of immobile nutrients, especially Zn, at acceptable levels (Ortaş and Akpınar, 2006; Smith and Read, 2008). In another study, *Fu. mosseae* inoculations increased nutrient uptake levels of horse bean plants (El-Sayad et al., 2002). Ortas (2012a) found that mycorrhizal inoculation significantly increased the horse bean P and Zn concentrations in three years under field conditions. With regard to general averages, the plants grown in unsterilized soils had higher nutrient concentrations than the plants grown in sterilized soils.

Table 4. Effects of different zinc doses and mycorrhiza inoculation on P, K, Fe and Zn concentration of horse bean plants grown in sterilized and unsterilized soils

			P	K	Fe	Zn
			%		mg kg^{-1}	
(-) Sterile	**(–M)**	**(0 mg kg^{-1}Zn)**	0.17 ±0.1^{a}	2.5 ±0.4^{a}	147.3 ±16.5^{bc}	36.0 ±0.0^{cd}
		(5 mg kg^{-1}Zn)	0.22 ±0.0^{a}	3.1 ±0.0^{a}	292.8 ±4.2^{a}	35.7 ±2.2^{cd}
	(+M)	**(0 mg kg^{-1}Zn)**	0.28 ±0.1^{a}	3.3 ±0.4^{a}	307.8 ±12.7^{a}	46.7 ±3.3^{ab}
		(5 mg kg^{-1}Zn)	0.23 ±0.1^{a}	3.9 ±0.9^{a}	308.4 ±62.6^{a}	41.0 ±3.0^{ac}
		General Average	0.23^{A}	3.20^{A}	264.1^{A}	39.9^{A}
(+) Sterile	**(–M)**	**(0 mg kg^{-1}Zn)**	0.15 ±0.0^{a}	2.6 ±0.2^{a}	153.7 ±57.7^{bc}	26.7 ±1.2^{d}
		(5 mg kg^{-1}Zn)	0.13 ±0.0^{a}	3.3 ±0.3^{a}	255.3 ±6.7^{ab}	33.5 ±1.5^{cd}
	(+M)	**(0 mg kg^{-1}Zn)**	0.19 ±0.0^{a}	3.1 ±0.3^{a}	254.2 ±18.6^{ab}	41.9 ±3.6^{a-c}
		(5 mg kg^{-1}Zn)	0.25 ±0.0^{a}	3.7 ±0.1^{a}	118.9 ±1.6^{c}	52.1 ±4.7^{a}
		General Average	0.18^{B}	3.18^{A}	195.6^{B}	38.6^{A}

± Standard error, P<0.05

The correlation analysis among the traits evaluated in this study is given in Table 5. As seen from the table, a positive relationship was found between shoot dry weight, P and Zn also between root dry weight and mycorrhizal dependency. Previous studies have found similar correlations between certain plant developmental parameters and mycorrhizal dependency (Azcon and Ocampo, 1981; Chalk et al., 2006). In the present study, Zn concentration was positively correlated with shoot dry weight, P, K concentration, root infection and negatively correlated with mycorrhizal dependency (Table 5).

Table 5. Correlation values of parameters evaluated in the study

Parameters	SDW	RDW	P	K	Fe	Zn	Inf.	MD
SDW	1	.430	.516*	.293	.474	.641**	.350	.098
RDW		1	.284	.409	.272	.277	.270	.717*
P			1	.542*	.333	.657**	.379	-.296
K				1	.431	.584*	.340	.074
Fe					1	.076	-.357	.716*
Zn						1	.764**	-.842**
Infection							1	-.650
MD								1

*: P <0.1, **: P < 0.05

SDW: Shoot Dry Weight; RDW: Root Dry Weight; P: Phosphorus; K: Potassium; Fe: Iron, Zn: Zinc; Inf: Root Infection; MD: Mycorrhizal Dependency

CONCLUSION

Present findings revealed that AMF, a beneficial fungi species and already existing in soils, had positive impacts on growth, development and nutrient uptake of horse bean plants. Such findings clearly indicated that mycorrhiza inoculations could play significant roles in growth and development of horse bean and similar plants grown especially in soils poor in plant nutrients. Also the results are shown that indigenous mycorrhizae have significant effect on plat growth and nutrient uptake as well. In this sense, agricultural strategies should be developed for naturally existing mycorrhiza in soils and thus for sustainable agricultural practices. Use of such natural rhizosphere mechanisms in agricultural practices will provide significant contributions to both the ecosystem and the economy of the countries largely dependent on foreign countries for chemical fertilizers. For sustainable agriculture, use of mycorrhiza as a biotical fertilizer will also provide significant contributions in reducing chemical fertilizer-induced environmental problems. Use of mycorrhiza in agricultural practices could also be recommended for healthy nutrition, food safety and quality.

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