



Mycorrhizae, elemental sulfur and phosphorus effects on pepper yield and nutrient uptake

Mikoriza, elementer kükürt ve fosforun biber verimi ve besin elementi alımı üzerine etkileri

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ÖZET / ABSTRACT

Aims: Fertilization contributes to the yield in agriculture. The objective of this study was to determine the effects of mycorrhizae and fertilization of phosphorus (P) and elemental sulfur (ES) on yield responses, root mycorrhizal inoculation level, root to shoot ratio and nutrient uptake of green pepper (*Capsicum annuum* L.).

Methods and Results: 100 mg kg⁻¹ ES and/or 100 mg kg⁻¹ P fertilizers were added with and without mycorrhizal inoculation into the soil. Green pepper was grown for 45 days on calcareous sterilized Karaburun soil. Root and shoot yield increased by mycorrhizal inoculation compared to the control treatment. While fertilization with ES or P alone resulted in yield increase in the non-mycorrhizal treatments, the reverse was the case in the mycorrhizal treatments. The combined effect of ES and P fertilization comparing P fertilization alone decreased the yield in the non-mycorrhizal treatments while the reverse was the case in the mycorrhizal treatments. Root to shoot ratios and shoot nutrient concentrations changed in both directions as independent from the yield and treatments. ES and/or P addition decreased insignificantly mycorrhizal inoculation level.

Conclusions: Obtained results indicated that ES and/or P fertilization affect yield of pepper, nutrient uptake, root to shoot ratio in both ways with insignificantly decreased root mycorrhizal infection level.

Significance and Impact of the Study: ES and/or P fertilization with mycorrhizal inoculation is important to get higher yield. For that reason, this study provides data about appropriate fertilizer or fertilizer combinations to prevent yield loss of pepper in mycorrhizal and non-mycorrhizal growth conditions.

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INTRODUCTION

Fertilization is common practice to increase yield in agriculture all over the world. For that reason various commercial fertilizers are applied in agricultural production system same as mycorrhizae as biological fertilizer.

Mycorrhizae (Mohammed et al., 2004; Al-Karaki, 1998), P (Antunes and Cardoso, 1991; Karaca, 2012a) and

elementel S addition (Karaca, 2012a) results in significant yield increases. Oxidation products of ES decreases soil pH that gradually increases solubility of plant nutrients for the plant uptake to result in higher growth. However, heavy metal concentration, if present, can increase by the decreased soil pH resulting in yield decrease (Karaca et al., 2013; Cui et al., 2004).

Combined treatment of ES and P fertilizers affect the yield in both ways. In that respect, it was reported that

ES and P combination compared to their individual addition significantly increased the root yield but the shoot yield remained unchanged (Karaca, 2012a). On the other hand, significant yield decreases were reported by ES and P combination compared to P addition alone (Karaca, 2012b).

There was no correlation all the time between the yield and shoot nutrient concentrations (Karaca, 2012a; Karaca, 2012b; Yibirin et al., 1996).

Mycorrhizal inoculation alone compared to the control treatment results in increased or did not change the root to shoot ratio. However, those ratios change in both directions in the case of ES and/or P additions (Karaca, 2012a; Karaca, 2012b). Accordingly, there are no correlations all the time between the yield and the root to shoot ratio (Karaca, 2014). Romero et al. (1986) proposed that there may be an optimum R: S ratio for plant growth.

The P fertilization to a P deficit soil decreases root mycorrhizal infection level, whereas ES treatment can compensate the decreased level resulted from the P fertilization (Karaca, 2012a).

A slight reduction on percentage of mycorrhizal colonization was noted with SO₂ (Diaz et al., 1996). This study evaluates the effect of ES and/or P on mycorrhizae for the yield, shoot nutrient concentrations for pepper in the loam textured, P deficient soil under greenhouse growth conditions.

MATERIAL and METHOD

Surface soil samples (0-30 cm) for Karaburun soil were taken from the non cultivated part of the Cukurova University experimental farm. The soils, Karaburun serial was a typic Xerorthent of the Entisol ordo in the Soil Taxonomy (Ozbek et al., 1974). The plot had not been cultivated for many years. Air dried soil samples were crushed, sieved (2 mm mesh opening) and autoclaved at 121°C for two hours prior to use as a growth medium. The pots surfaces were sterilized with ethanol 96 % (v/v), washed by distilled water and dried out prior to the use. 4 kg of autoclaved soil were placed in the plastic pots and following treatments were made;

MoPoSo: Control treatment in which soil amended with 500 mg kg⁻¹ N (as urea), 250 mg kg⁻¹ K (as KNO₃), 5 mg kg⁻¹ Zn (as ZnSO₄) and 20 mg kg⁻¹ Fe (as Fe-EDDHA) and then soil samples were thoroughly mixed to have homogenous distribution of nutrients.

MoPoS+: Control + 100 mg kg⁻¹ ES.

MoP+So: Control + 100 mg kg⁻¹ P (as triple super phosphate).

MoP+S+: Control + 100 mg kg⁻¹ P + 100 mg kg⁻¹ ES.

M+PoSo: *Glomus mossea* AM fungi type as the mycorrhizae (as 145 g soil taken from the vicinity of the dead vineyard roots at the University Farm for the average 1000 spore/pot inoculation) was added to the control treatment. The mycorrhizal density of soil was determined by the method of Gerdemann and Nicolson (1963).

M+PoS+: Control + the mycorrhizae + 100 mg kg⁻¹ ES.

M+P+So: Control + the mycorrhizae + 100 mg kg⁻¹ P.

M+P+S+: Control + the mycorrhizae + 100 mg kg⁻¹ P + 100 mg kg⁻¹ ES.

All fertilizers were mixed thoroughly in the soil to have homogenous distribution. However, the mycorrhizal inoculum was mixed into the top 5 cm of the soil. Following the addition of the inoculum, 1000 ml water was added to the each pot to bring the soil about field capacity and allowed to drain for 5 days.

Commercial green pepper seeds (*Capsicum annum* L.) were sown into sterilized growth medium of soil and organic matter mixture (soil/organic matter: 2/1 (v/v)) and grown for 35 days. The seedlings were carefully extracted from the nursery and transplanted into the pots in the same greenhouse and irrigated when required. The seedlings grew for one and half month. The plants were harvested by cutting just above the soil surface and the shoots were separately dried at 75°C to a constant weight after clearing possible contaminants by tap water and then distilled water respectively. Plants dry were grinded to a particle size below 0.5 mm to obtain homogenous alicot.

Nitrogen (N) content of samples was determined by Kjeldahl digestion and steam distillation (Lees, 1971). For determination of other nutrient elements samples were digested in HNO₃ and H₂O₂ (v/v: 4/1) mixture (Cem, MarsXpress Manual). Phosphorus concentration of the digests were colorimetrically determined (Shimadzu 1201 model UV/VIS spectrometer) according to Murphy and Riley (1962) and potassium (K), sodium (Na), calcium (Ca), magnesium (Mg), iron (Fe), copper (Cu), manganese (Mn) and zinc (Zn) concentration were determined using ICP-AES (Varian, Liberty Series II) according to Kacar (1972).

After separating from the soil, the fresh roots were washed under running tap water, followed by distilled water and dried on tissue paper. Then the root biomasses were determined. Fine roots were freshly preserved in a mixture (250:13:15 v/v) of ethanol, glacial acetic acid and formalin (Ortas et al., 2004) until the determination of mycorrhizal infection. The root clearing and staining procedure and the degree of mycorrhizal infection in the root cortex was assessed by the method of Koske and Gemma (1989).

Basic physical and chemical properties of autoclaved soil were analyzed as follows: soil texture analysis by a hydrometer (Bouyoucos, 1951), organic matter by using Lichterfelder wet ashing (Schlichting and Blume, 1966), soil reaction and electrical conductivity by means of a combined electrode and EC meter in saturation paste, respectively (Schlichting and Blume, 1966), Ca carbonate equivalent by a manometric method (Loeppert and Suarez, 1996), cation exchange capacity (CEC) by saturating sodium acetate (1M pH 8.2) and then replacing the Na with ammonium acetate (1 M pH 7.0) (U.S. Salinity Laboratory Staff, 1954), available phosphorus by Olsen method (Olsen et al., 1954), total nitrogen (N) by Bremner (1996), soil nitrate by Fabig (1978), soil ammonium by Fachgruppe Wasserchemie in der Gesellschaft Deutscher Chemiker (1983), exchangeable potassium (K) with neutral ammonium acetate by Pratt and Morse (1954), DTPA extractable microelements (Fe, Zn, Cu and Mn) by Lindsay and Norvell (1978), soil density by a picnometer by Blake and Hartge (1986b), bulk density by Blake and Hartge (1986a) and permeability by a constant head permeameter by Klute and Dirksen (1986).

The Karaburun soil series is classified as a clay loam textured soil (sand 312.5 g kg⁻¹; silt 390.2 g kg⁻¹; clay 297.3 g kg⁻¹). The pH of the soil is slightly alkaline (7.44) and there is no salinity problem (EC = 0.85 < dSm⁻¹). The organic matter content is low (14.2 g kg⁻¹), while the CEC is 43.71 cmol kg⁻¹, density is 2.67 g cm⁻³; bulk density is 1.456 g cm⁻³; porosity 45.4% calculated from bulk density and density; and the permeability is 2.8 cm h⁻¹(medium). The plant nutrients of the soil are low: P 4.48 mg kg⁻¹; K 140 mg kg⁻¹; NH₄ 3.86 mg kg⁻¹; NO₃ 1.85 mg kg⁻¹; Fe 0.145 mg kg⁻¹; Cu 0.084 mg kg⁻¹; Mn 0.478 mg kg⁻¹ and Zn 0.125 mg kg⁻¹. Soil is very calcareous with 425 g kg⁻¹ CaCO₃ content.

The data were subjected to the analysis of variance using MSTAT-C statistical analysis package (MSTATC, Michigan State University, East Lansing, MI, USA). The mean separation was made by Least Significant Difference (LSD) test at p = 0.05.

RESULTS and DISCUSSION

Shoot and Root Yield, and Shoot Nutrient Concentrations

MoPoS+ treatment compared to the MoPoSo treatment increased shoot and root biomasses (Table 1, Figure 1) being in parallel with the previous findings (Karaca, 2012a) with increased shoot P and Mg concentration and decreased shoot Fe and Mn concentration (Figure 2,3). Those yield increases can be attributed to solubilizing

effect of ES fertilization for the nutrients in a growth medium. Similar yield increases were obtained by MoP+So treatment compared to the control treatment being in line with the previous findings (Antunes and Cardoso, 1991) accompanied by the increased shoot P, Ca, Mg, Fe, Zn and Mn concentrations and decreased shoot Cu concentration. Those yield increases can be expected by P fertilization, some to a great extent, in P-deficient soils. On the other hand, MoP+S+ treatment compared to MoP+So treatment significantly decreased the shoot and root yield (Table 1 and Figure 1) being consistent with the previous findings (Karaca, 2012b; Cui et al., 2004) with the increased shoot Ca, Mg, Mn concentrations and decreased shoot P, Fe, Zn and Cu concentrations (Figure 2,3). Those yield decreases can be resulted in excessive accumulation of Mn and nutrient imbalances in shoots depending on the S-induced pH decreases in the rhizosphere. Thus, there is no correlation all the time between the yield and shoot nutrient concentrations among the treatments. Shoot nutrient concentrations can independently change in both directions due to more complex interactions of nutrient elements in soil and different plant tissue (Karaca, 2012a; Karaca, 2012b; Yibirin et al., 1996).

M+PoSo treatment compared to MoPoSo treatment significantly increased shoot and root yield (Table 1 and Figure 1) being in line with the mycorrhizal infection (Mohammed et al., 2004; Al-Karaki, 1998) that resulted in the increased shoot P, Mg, Ca, Zn, Mn concentrations and decreased shoot Fe and Cu concentrations (Figure 2,3). Those yield increases can be attributed to enhanced nutrient uptake by the plant root infected with mycorrhizae. Mycorrhizal infection increases the root-surface contact surface area which enable to enhanced nutrient uptake. On the contrary, M+PoS+ treatment compared to the M+PoSo treatment significantly decreased the shoot and root yield due possibly to excessive uptake of Fe, Zn and Mn and reduced concentrations of P, Ca, Mg and Cu in plant tissues. In a similar way, it was reported that such yield decrease may be related to the mimic of heavy metal toxicity (Cui et al., 2004; Karaca, 2014).

Similar yield decreases were obtained by M+P+So treatment compared to the M+PoSo treatment. This fact is indicating that yield decreases was caused by the antagonistic relation between mycorrhizae and phosphorus fertilization. On the other hand, there were significantly higher accumulation of P, Ca, Mg, Fe, Zn and Mn with reduced Cu concentrations in plant tissue for the M+P+So treatment compared to M+PoSo treatment (Table 1 and Figure 2,3). That yield decrease is in accordance with the previous findings (Cui et al., 2004;

Karaca, 2014) and indicating an accumulation effect due possibly to deficiency of any other element.

M+P+S+ treatment compared to M+P+So treatment increased the shoot and root yields in the mycorrhizal treatments. Those yield increases can be attributed to differences of the shoot nutrient concentrations, too.

Thus, the shoot P concentration significantly increased while the shoot Ca, Mg, Fe, Zn and Mn concentration significantly decreased for the M+P+S+ treatment compared to the M+P+So treatment. However, the shoot Cu concentration was remained constant.

Table 1. Response of pepper to different treatments in Karaburun soil

Treatment	Shoot DW (mg)	Root DW (mg)	R/S	Mycorrhizal infection (%)	N (mg kg ⁻¹)	P (mg kg ⁻¹)	K (mg kg ⁻¹)
MoPoSo	241.67e	25.00d	0.10c	0.00	46783.33	1533.33g	16018.07
MoP+So	1183.33a	213.33a	0.18ab	0.00	46523.33	2566.67a	18351.76
MoPoS+	282.33de	28.67cd	0.10c	0.00	47496.67	1866.67e	16187.49
MoP+S+	1058.33b	145.00b	0.14bc	0.00	47593.33	2166.67c	15500.70
M+PoSo	413.33c	37.33c	0.09c	56.67	44453.33	2033.33d	17989.11
M+P+So	333.33d	28.33cd	0.09c	40.00	48056.67	2133.33c	18711.09
M+PoS+	338.33d	32.67cd	0.10c	36.67	46283.33	1766.67f	19035.90
M+P+S+	1050.00b	211.00a	0.20a	6.67	47140.00	2266.67b	17341.28
F Value	214.2652	123.864	169.3602	2.4889 (0.137)	1.4984	345.0378	0.9948
(Prob.)	(<0.0001)	(<0.0001)	(<0.0001)		(0.2411)	(<0.0001)	
LSD	70.14	11.18	0.0554	9.063	2719	65.43	649.3

* Different letter implies significant differences in the same column.

Table 1. Continued

Treatment	Ca (mg kg ⁻¹)	Mg (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Zn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Na (mg kg ⁻¹)
MoPoSo	6777.00f	4771.37e	161.48ef	121.38e	21.04a	126.89d	2588.59
MoP+So	7043.47e	4856.87de	296.47c	134.08d	15.30bc	154.45bc	1762.75
MoPoS+	6577.43f	5038.33cde	143.77fg	118.51e	21.61a	108.68e	2604.79
MoP+S+	7809.67bc	5228.53bc	177.10de	120.64e	13.34d	159.14ab	1661.00
M+PoSo	7642.73c	5322.00b	129.74g	144.19c	15.56b	150.16c	1942.67
M+P+So	8541.70a	5680.60a	458.68a	188.93a	12.87d	164.64a	1663.25
M+PoS+	7345.13d	5262.57bc	439.00b	167.14b	14.62c	153.54bc	1816.71
M+P+S+	7966.23b	5073.73bcd	184.67d	120.22e	13.27d	122.84d	1474.50
F Value	25.4586	6.5388	712.6051	256.7127	19.4614	93.8794	0.1807
	(0.0002)	(0.0228)	(<0.0001)	(<0.0001)	(0.0006)	(<0.0001)	
LSD	264.3	273.5	19.35	5.428	0.9398	7.535	139.1

* Different letter implies significant differences in the same column.

There were no correlations all the time between the yield and shoot nutrient concentrations due to accumulation- and dilution -effect. In that respect, higher yields compared to lower yields can show increased, decreased or stable shoot nutrient concentrations for any nutrient independent from the others (Table 1 and Figure 1,2,3). There are some reports

pointing out such treatment induced ambiguities in nutrient concentrations (Karaca, 2014; Karaca, 2012a; Karaca, 2012b; Yibirin et al., 1996).

Those results above indicate that different treatments in the growth media affect the efficient use of nutrients and mycorrhizae with the subsequent yield differences.

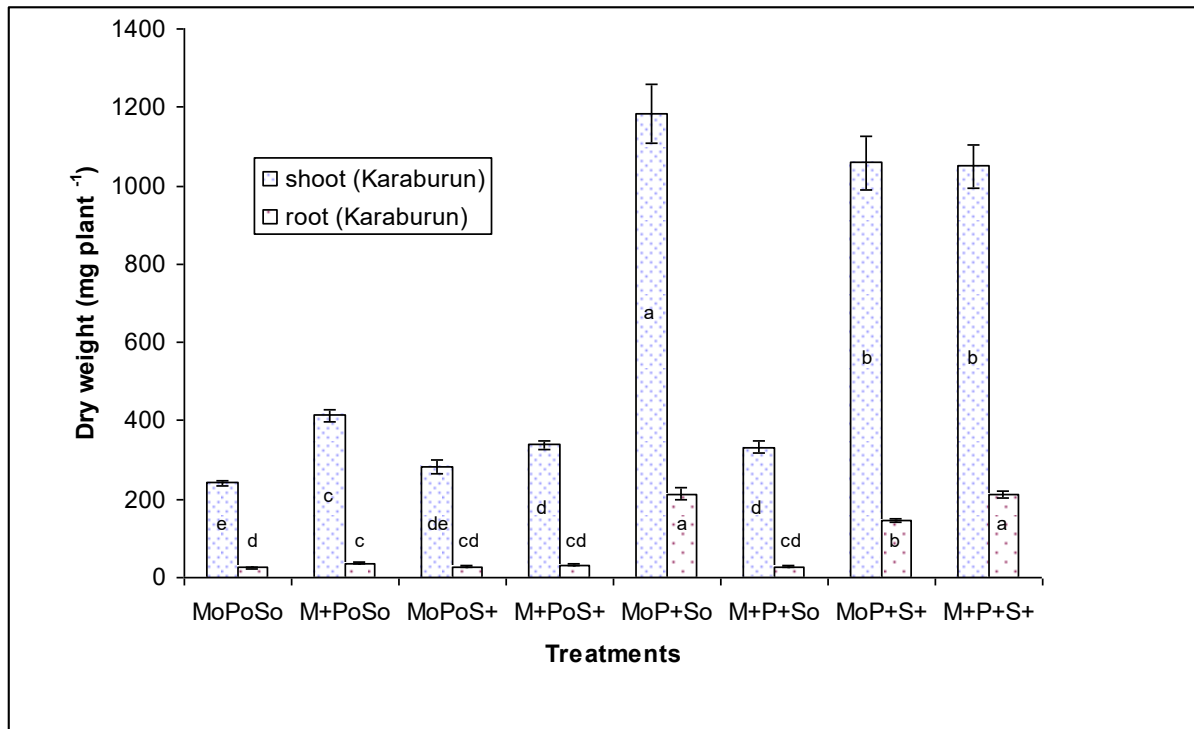


Figure 1. Pepper shoot and root dry weight biomasses. Different letters indicate significant difference between the treatments. Error bars indicate standard deviation.

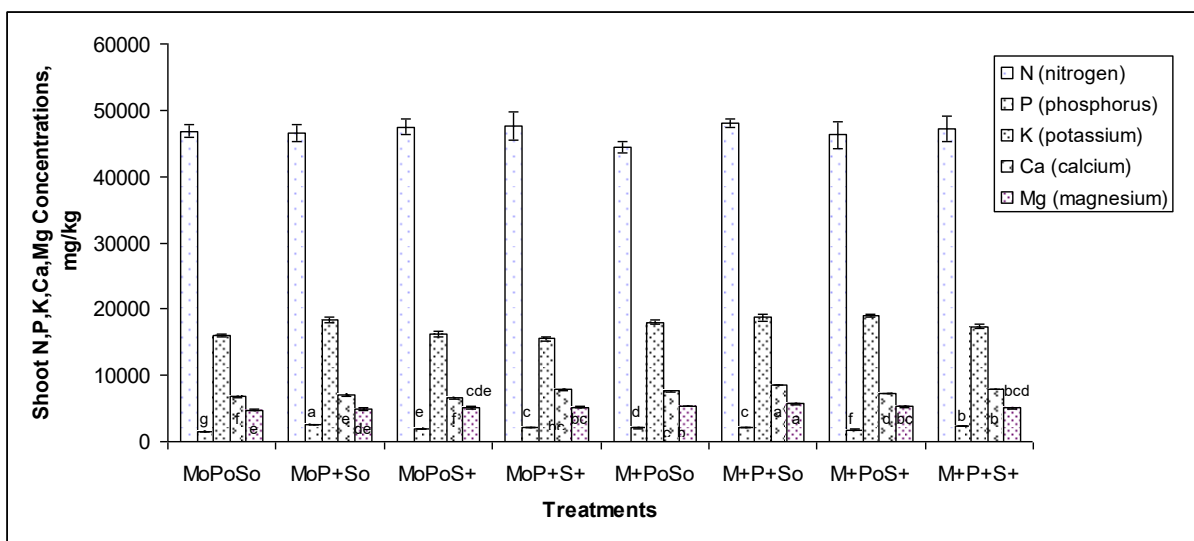


Figure 2. N, P, K, Ca, Mg concentrations of pepper shoots. Different letters indicate significant difference between the treatments. Error bars indicate standard deviation.

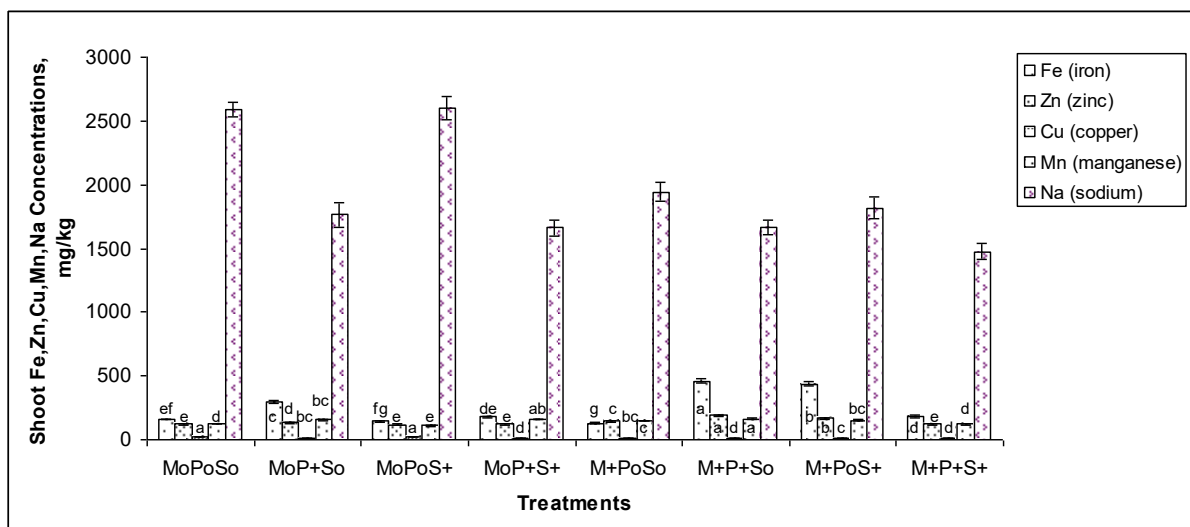


Figure 3. Fe, Zn, Cu, Mn, Na concentrations of pepper shoots. Different letters indicate significant difference between the treatments. Error bars indicate standard deviation.

Root Mycorrhizal Infection Responses to ES, P and Mycorrhizal Inoculation

None of the non-inoculated plants exhibited any mycorrhizal structures (Table 1 and Figure 4). In the mycorrhizal treatments, the highest root mycorrhizal infection level was obtained by M+PoSo treatment as 56.67%. Phosphorus supplement with mycorrhizae reduced the infection ratio to 40% that similar responses were also reported by Karaca (2012a). Sulfur addition also resulted in similar decreasing trend (36.7%) as observed for M+PoS+ treatment (Figure 4) which is not consistent with the previous findings (Diaz et al., 1996).

Nevertheless, as small as 6.67% mycorrhizal infection was recorded for M+P+S+ treatment that yielded maximum crop biomass. Those findings indicate that individual and co-effect of P and ES treatments have negative effects on mycorrhizal infection but only their individual effects resulted in yield decreases. Those yield decreases were compensated to a great extent by getting decreased of root mycorrhizal infection percentage to the lowest level (Table 1 and Figure 1,4) being not consistent with the previous findings (Karaca, 2012a).

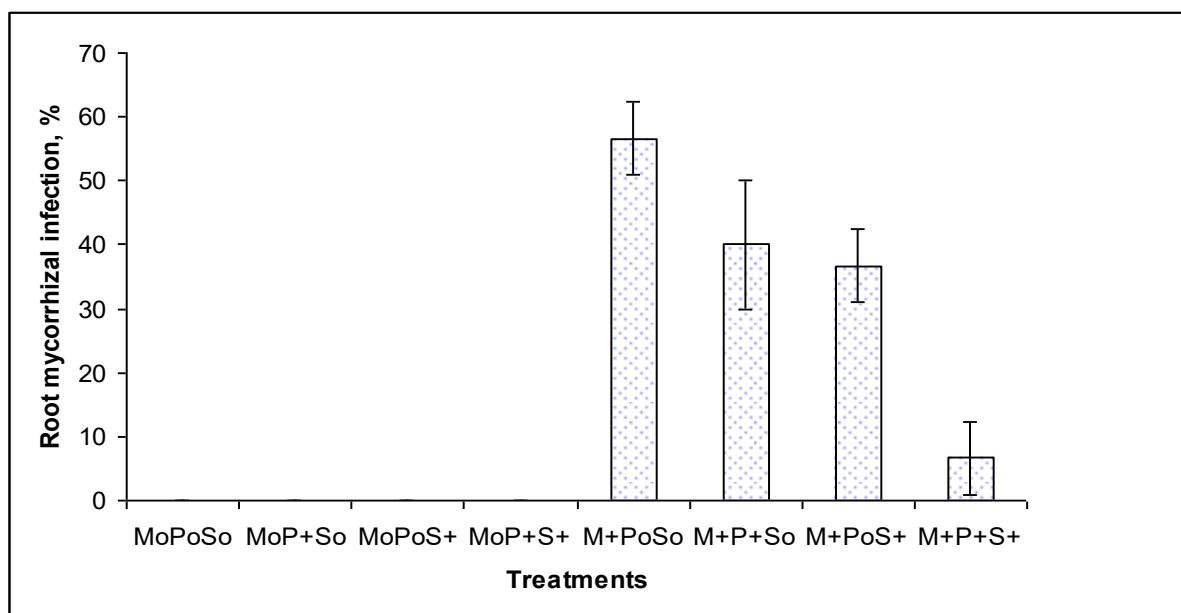


Figure 4. Treatment induced mycorrhizal infection percentages. Different letters indicate significant difference between the treatments. Error bars indicate standard deviation.

Root : Shoot ratio

M+PoSo treatment compared to the MoPoSo one did not affect the root to shoot ratio. On the other hand, the ratios responded in both directions among the other all treatments. Those findings are similar to the findings (Karaca, 2012a; Karaca, 2014) who reported that root: shoot ratio decreases or remains around at the same level between mycorrhizal inoculation alone and control treatment but root to shoot ratio changes in both directions by either ES or P fertilizations. Moreover,

there were no straightforward relations between yield and root to shoot ratio (Karaca et al., 2013). Accordingly, higher yield compared to lower one may have higher, lower or constant root to shoot ratio as presented in Table 1 and Figure 1 and 5. Those changed ratios may lend support also to the hypothesis (Romero et al., 1996) who proposed that there may be an optimum R: S ratio for plant growth. The other point is when the plant have readily accessed to plant nutrient promote shoot growth rather than the root growth.

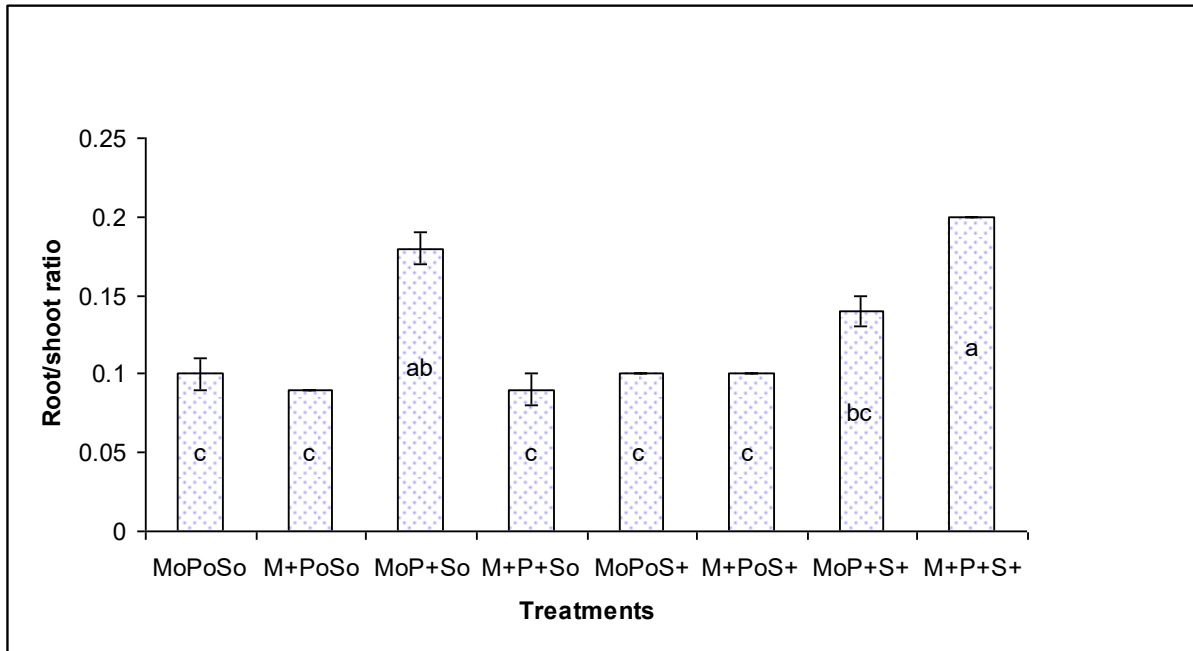


Figure 5. Root to shoot ratio (dry weight) of pepper plant under different treatments. Different letters indicate significant difference between the treatments. Error bars indicate standard deviation.

CONCLUSIONS

Yield increases were obtained by P addition same as ES addition in the non-mycorrhizal treatments. The highest yield was obtained by P addition alone. That highest yield level decreased by combination effect of ES and P addition indicating antagonistic effect between ES and P. While mycorrhizal inoculation increased yield, both P addition alone and elemental S addition alone resulted in significant yield decreases in the mycorrhizal treatments indicating the negative effect of ES or P on mycorrhizae. On the other hand, ES and P addition in combination resulted in the highest yield in the mycorrhizal treatments as response to the lowest root mycorrhizal infection level. Phosphorus, Ca, Mg, Fe, Mn, Zn and Cu shoot concentrations independently changed from each other in both direction.

ES or P addition decreased insignificantly root mycorrhizal infection level while root to shoot ratios

changed in both directions.

From all above it can be concluded that P and/or ES additions require to be regulated well in the changing growth medium conditions to get higher yield in agricultural production system.

ÖZET

Amaç: Gübreleme tarımda verime katkı sağlar. Bu çalışmanın amacı yeşil biber (*Capsicum annuum L.*) verimi, kök/sürgün oranı, besin elementi alımı kökle mikorizanın infekte olma düzeyi üzerinde mikoriza ve elemental kükürt (ES) ve fosfor (P) gübrelemesinin etkisini belirlemektir.

Yöntem ve Bulgular: 100 mg/kg ES and/or 100 mg/kg P gübreleri mikorizalı ve mikorizasız toprağa ilave edildi. 45 gün süreyle yeşil biber bitkisi kireçli sterilize edilmiş Karaburun toprağında yetiştirildi. Kök ve sürgün verimi mikoriza aşılması ile kontrol uygulamasına göre arttı.

Tek başına ES yada P gübrelemesiyle mikorizasız uygulamalarda verim artarken, mikorizalı uygulamalarda tersi durum söz konusu idi. ES and P gübrelemesi birlikte tek başına P gübrelemesine kıyasla mikorizasız uygulamalarda verimi düşürürken, mikorizalı uygulamalarda tersi durum söz konusu idi. Kök sürgün oranı ve sürgün besin elementi konsantrasyonu verimden ve uygulamalardan bağımsız olarak iki yönlü değişim gösterdi. ES ve/veya P ilavesi mikorizal infekte olma düzeyini önemsiz düzeyde azalttı.

Genel Yorum: Elde edilen bulgular gösteriyor ki ES and/or P gübrelemesi kökle mikorizanın infekte olma düzeyini önemsiz ölçüde azaltmakla birlikte yeşil biber verimini, besin elementi alımını, kök sürgün oranını iki yönde etkilemektedir.

Çalışmanın Önemi ve Etkisi: ES and/or P gübrelemesi mikoriza aşılmasıyla birlikte daha yüksek ürün elde etmek için önemlidir. Bu nedenle bu çalışma biberde verim kayıplarını önlemek için mikorizalı ve mikorizasız yetiştirme koşullarında uygun gübre ve gübre kombinasyonları hakkında bilgi sağlamaktadır.

Anahtar Kelimeler: Mikoriza, elementel kükürt, fosfor, sürgün element konsantrasyonları, biber verimi.

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CONFLICT OF INTEREST DECLARATION

The author(s) declare no conflict of interest for this study.

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