Original article



Nutrient uptake-modification of different plant species in Mediterranean climate by arbuscular mycorrhizal fungi

M. Rafique^{1,2} and İ. Ortas¹

¹ Department of Soil Science and Plant Nutrition, University of Çukurova, Faculty of Agriculture, Adana, Turkey ² Department of Plant Sciences, Quaid-I-Azam University, Islamabad, Pakistan

Summary

Soil fertility in the semi-arid Mediterranean region is poor and mycorrhizal inoculation could be an important agricultural strategy for reducing fertilizer use and also increase the food quality. To evaluate the substantial reliance of selective horticultural crops on arbuscular mycorrhizal (AM) fungi, a field experiment was conducted in the Mediterranean coastal region of Turkey. Under field conditions, pepper (Capsicum annuum L.), tomato (Lycopersicon esculentum Mill.), eggplant (Solanum melongena L.) and cucumber (Cucumis sativus) were inoculated with Funneliformis mosseae and Claroideoglomus etunicatum at two levels of P₂O₅. Yield enhancement, inoculation effectiveness and nutrient concentration in the plants were assessed. Root colonization significantly improved in mycorrhizal inoculated plants by 51-80% irrespective of P₂O₅ application. Similarly, significant impact of P₂O₅ and AM fungi was found for pepper and eggplant yield increase as 8-35% and 6-44% respectively. Phosphorus percentage in plant tissues was significantly different in tomato plant (0.22-0.30%) and AM fungi increased its concentration also in other crops. AM fungi application strongly influenced nutrients concentration and improved the plant quality in all studied crops against control. Under field conditions, AM fungi with P205 application is best responding for yield enhancement and nutrient uptake leading to a quality food. Therefore, plant growth and nutrient uptake is strongly dependent on AM fungi inoculation moreover to reinstate soil quality. Our results suggested that application of mycorrhizal fungi in plant growth improvement is inevitable under Mediterranean climate.

Keywords

Claroideoglomus etunicatum, cucumber, Funneliformis mosseae, mycorrhizae, phosphorus, tomato

Introduction

Arbuscular mycorrhizal (AM) fungi form symbiotic association with plants such as horticultural and cash crops (Ortas et al., 2008). As they are acquiring more importance regarding dietary and economic aspects, their reliance on AM fungal inoculation strengthens its application. Pepper (*Capsicum* spp.) with AM fungal inoculation highlights notable changes in nutritional, biochemical and physiological traits of plants (Pereira et al., 2016).

Significance of this study

What is already known on this subject?

 Use of AM fungi in plant growth, P-uptake is evaluated in greenhouse studies. Its broad scale application in the field is becoming a new approach for horticultural crops.

What are the new findings?

 This study shows modification of nutrient uptake pattern in AM fungal inoculated horticultural crops, under changing climatic conditions of the Mediterranean region.

What is the expected impact on horticulture?

 Impact of AM fungi on horticultural plants grown under field conditions and changing climate is observed for organic farming regarding yield and quality.

The symbiotic root-fungal association belowground is postulated for plant growth promotion and nutrient uptake, particularly phosphorus (P) and zinc (Zn) as essential but immobile nutrients. Mycorrhizal inoculation increases growth of tomato, pepper (Colla et al., 2015), eggplant (Ortas et al., 2003), watermelon and cucumber (Ortas, 2010) under limited P availability. Inoculated pepper seedlings flower earlier than non-inoculated with high concentration of P and Zn. By AM fungal inoculation, P concentration in shoot and plant biomass increases 73 to 95% (Tawaraya et al., 2001). Similarly, P concentration is augmented in tomato plant tissues (Hart et al., 2015). Pepper plants inoculated with Funneliformis mosseae improved mycorrhizal dependency by 48% (Latef and He, 2014). However, use of AM fungi with different doses of fertilizer reduces AM fungal abundance in high-dose treatment (Chen et al., 2012).

Additionally, tomato has a high amount of macronutrients, particularly P, Mg, Ca, and trace elements such as Zn, Cu, Mn, and Fe (Dorais et al., 2008). In a study on tomato, 5 different AM fungal species were used, mycorrhizal dependency, P, and Zn uptake were increased while their biomass was also more than non-inoculated seedlings. Similarly, 51% AM fungal colonization with low P availability was observed in tomato (Kowalska et al., 2015).

Plant symbiosis for mycorrhizal inoculation is strongly linked with soil fertility and fertilization (P availability). In P-deficient soils, yield is dependent on mycorrhizal effectiveness influenced by cultivar (Khalil et al., 1999), ecotype (Kormanik et al., 1977), soil P (Ortas et al., 2003) and mycorrhizal species (Ortaş, 2010). In Mediterranean climate, horticul-



tural crops have key importance in the agricultural system. Climatic changes and nutrients removal from soil, depleting soil quality and affecting plant yield. In the Mediterranean region, AM fungi application is proposed to be more vital for horticultural seedlings growth, but a smaller number of studies have been conducted primarily in field conditions.

In this study, 4 different plants such as tomato, pepper, eggplant, and cucumber were inoculated by mycorrhizal species under two levels of P_2O_5 as -P × *E. mosseae*, -P × *C. etunicatum*, +P × *F. mosseae*, and +P × *C. etunicatum*. We hypothesized that, due to environmental changes, AM fungi colonization, yield, and nutrient uptake-modification would depend on the host plant. Objectives of the study were to evaluate inoculation effectiveness, yield and nutrient uptake-modification in field condition of Mediterranean climate for a continuous long-term experiment on changing climate.

Materials and methods

Experimental setup

In 2005, the experiment was carried out in the Menzilat soil series (Typic Xerofluvents Fluvents, Entisols by USDA classification) located at the Research Farm of the Çukurova University (37°00'54.31"N, 35°21'21.56"E; 31 m above mean sea level) in the Mediterranean region of Adana, Turkey. Annual air temperature is 19.1°C and precipitation of 670.8 mm whereas 75–80% of the annual precipitation is received between November and April, with a mean annual humidity of 60–70 %. Some soil properties are presented in Table 1.

Non-mycorrhizal seedlings were produced under glasshouse conditions in a sand-soil-compost (7:2:1 v/v) growth medium. The substrate was autoclaved at 121°C for 2 h before use as a growth media. Seedlings of 4 leaves were transplanted to field, and approximately 500 AM fungal spores were added 50 mm below seedlings, and non-mycorrhizal plants received the same amount of AM fungal spore-free medium filtrate to balance microbial community. A complete randomized block design with three replications was used, in each block, the main treatments, -P (0 kg P_2O_5 ha⁻¹) and +P (100 kg P_2O_5 ha⁻¹) were applied with two different mycorrhizal inoculation as F. mosseae and C. etunicatum (Schüßler and Walker, 2010) as -P × F. mosseae, -P × C. etunicatum, +P × F. mosseae, and +P × C. etunicatum. Growth of various plant species such as tomato (Lycopersicon esculentum Mill.), (cv. SC2121), pepper (Capsicum annuum L.), (cv. Kahramanmaras), eggplant [aubergine] (Solanum melongena L.) (cv. Pala), and cucumber

TABLE 1. Chemical characteristics of the Menzilat soil at theexperimental site.

Parameters	Units	Values
Clay	(%)	37.50 (±2.49)
Silt	(%)	30.96 (±0.10)
Sand	(%)	31.53 (±2.57)
CEC	(meq. 100 g ⁻¹)	7.54 (±0.43)
CaCO ₃	(%)	32.50 (±2.20)
рН	(1:2.5 w/v)	7.87 (±0.04)
$WAS_{1-2mm^{\star}}$		69.9 (±1.52)
Total C	(%)	4.83 b (±0.06)
Total N	(%)	0.140 (±0.01)
Total P	(mg kg⁻¹ soil)	10.47 (±1.86)

*WAS_{1-2mm}: wet aggregate stability of 1-2 mm aggregates measured only in autumn.



(*Cucumis sativus* L.), (Yayla F_1 local variety) were compared under field conditions. The plot for each plant species was 15 m², and it was amended with a base fertilization of 200 kg N as NH₄NO₃ ha⁻¹. It was supplied as split dose, half of N at the beginning of sowing and the remaining half was applied before the flowering stage. Each plant species was considered as separate experiment according to ecological growth period. Specific plant × plant and row × row distance was different for plants as eggplant (15 cm, 40 cm), pepper (10 cm, 40 cm), tomato (15 cm, 40 cm) and cucumber (20 cm, 50 cm) whereas number of plants sampled for each crop in 6 m⁻²-area were 10, 15, 10, and 6, respectively.

Mycorrhizal colonization and inoculation effectiveness

Before flowering of the plants, roots were removed and carefully washed for assessment of mycorrhizal colonization. Standard procedure for root cleaning and staining was followed (Kosola et al., 2007). AM fungal colonization percentage was calculated under a stereo microscope out of 100 root segments (Gioannetti and Mosse, 1980). On harvesting, fresh weight and dry weight yield was recorded. Inoculation effectiveness (IE) of AM fungal inoculated plant yield was calculated for each species based on formula:

Inoculation effectiveness (IE) =
$$\frac{[Yield (+M) - Yield (-M)]}{Yield (+M)} \times 100$$

where +M = inoculated plants, and -M = non-inoculated plants.

Nutrient concentration and statistical analysis

Before each crop flowered, top leaves of plant were collected (Benton, 1998) for nutrient analysis. Plant leaves were oven-dried at 65°C for 48 h and ground with material feed size <8 mm using a Tema mill (Retsch Solutions in Milling and Sieving), and 0.2 g of plant material was ashed at 550°C and dissolved in 3.3% HCl. Leaf P concentration was determined with the vanadate-molybdate yellow colorimetric method by spectrophotometer while Zn concentration was determined by atomic absorption spectrometry (Chapman and Pratt, 1962).

All data were statistically analyzed using the analysis of variance (ANOVA) procedure in SAS 9.1 (2009) program to assess the effect of fertilizer and mycorrhizal inoculation on plant quality and yield parameters. Least significance differences (LSD) at p = 0.05 were tested to determine the significant differences among treatment means.

Results

Mycorrhizal colonization

Cucumber plant showed more root colonization for $-P \times E$ mosseae as 67% while $+P \times E$ mosseae, it was observed as 59.7%. Root colonization was observed significant (p<0.001) in cucumber plant. Treatments of $-P \times E$ mosseae and $-P \times C$. etunicatum showed more root colonization than treatments of $+P \times E$ mosseae and $+P \times C$. etunicatum. As, in pepper, both AM fungi significantly (p=0.001) infected the plants by 67%. In tomato and eggplant, *E* mosseae and *C*. etunicatum vas observed in the $+P \times E$ mosseae and $+P \times C$. etunicatum treatments 52.5% and 53.8% respectively. Similar trend was 59.8% and 60.8%. Root colonization for the tomato and eggplant was observed significant (p<0.001) (Figure 1).



FIGURE 1. Mycorrhizal root infection against different doses of phosphorus application.



FIGURE 2. Plant yield in kg m⁻² against different doses of phosphorus application.

Plant yield

In experiment, +P has significant (p=0.005) effect on pepper yield. Maximum yield of 3.2 kg m⁻² was obtained for pepper in -P × *F. mosseae* application. Similarly, -P × *C. etunicatum* application produced 1.9, 5.1 and 1.2 kg m⁻² of tomato, eggplant, and cucumber respectively. Besides that, in P₂O₅ applied treatments, *C. etunicatum* produced yield of 2.5 kg m⁻² in pepper and 5.8 kg m⁻² in eggplant while for the tomato and cucumber, *F. mosseae* was prominent by producing 1.8 and 1.3 kg m⁻² yield respectively (Figure 2).

Leaf nutrient concentration

Concentration of P was observed more in AM fungal inoculated plants of *Solanaceae* family irrespective of P_2O_5 application. Observed P in mycorrhizal inoculated pepper, tomato and eggplant was 0.27–0.31% while in cucumber it was 0.22–0.23%. Among all the plants, tomato exhibited statistically significant P in leaves (p < 0.02) while contribution of mycorrhization was significant in pepper (p < 0.006) and tomato (p < 0.008) in Tables 3 and 4.

Presence of Zn in leaves was 19.9–27.9 mg kg⁻¹ for mycorrhizal inoculated plants of *Solanaceae* family whereas in cucumber it was 28.3–33.9 mg kg⁻¹ except for $-P \times C$. etunicatum which showed 14.9% concentration. Besides that, pepper with +P × Mycorrhizae decreased Cu concentration whereas with -P × Mycorrhizae, Cu concentration was increased (p<0.05) (Table 3). F. mosseae and C. etunicatum ensured better uptake of Cu in contrary to non-inoculated plants. AM fungal application significantly (p < 0.0001) improved Fe uptake in tomato leaves. Similarly, with +P, eggplant leaves had significant (p < 0.05) concentration of Fe in comparison to other plants. Tomato plants with F. mosseae showed significantly high concentration of Fe irrespective to the P. Tomato plants with -P × Mycorrhiza significantly (p < 0.05) favoured Fe uptake than tomato plants with +P × Mycorrhiza (Table 3). F. mosseae inoculation increased Mn concentration than *C. etunicatum* in pepper plants irrespective of P doses. Similarly, Mn concentration was insignificantly changed in tomato and cucumber plants among all the treatments. Besides that, -P × F. mosseae showed significant increase in Mn concentration.

Interestingly, results highlight AM fungi application in the field and its commercial use for sustainable agriculture in future. Data of observed parameters varied by application of both AM fungi, which shows its specificity to the plants (Table 2).

TABLE 2.	Yield increase and	inoculum eff	ficiency efficienc	v of all plant	ts under different	P doses.

Plant	Source		Yield increase (%)	Mycorrhizal inoculum efficiency (%)
Pepper	-P	F. mosseae	35.7	26.3
		C. etunicatum	20.0	16.7
	+P	F. mosseae	8.0	7.4
		C. etunicatum	22.6	18.4
Tomato	-P	F. mosseae	37.8	27.4
		C. etunicatum	76.1	43.2
	+P	F. mosseae	29.1	22.6
		C. etunicatum	6.8	6.4
Eggplant	-P	F. mosseae	10.6	9.6
		C. etunicatum	44.0	30.6
	+P	F. mosseae	6.4	6.0
		C. etunicatum	20.1	16.7
Cucumber	-P	F. mosseae	42.6	29.9
		C. etunicatum	69.3	40.9
	+P	F. mosseae	83.2	45.4
		C. etunicatum	41.3	29.2

Mean of three replicates; F: Funneliformis, C: Claroideoglomus.



Plant	Sour	ces	P (%)	Zn (mg kg ⁻¹)	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)
Pepper	-P	Control	0.23 ±0.03c	18.90 ±1.85c	7.95 ±0.8d	201.40 ±3.96a	99.7 ±1.4c
		F. mosseae	0.30 ±0.01a	20.10 ±0.71bc	13.3 ±0.6bc	210.40 ±16.69a	114.7 ±1.4ab
		C. etunicatum	0.29 ±0.02ab	19.93 ±1.96bc	13.5 ±1.1b	190.05 ±1.63a	108.7 ±3.7a-c
	+P	Control	0.26 ±0.01bc	20.20 ±0.42bc	19.2 ±1.5a	255.30 ±17.68a	119.8 ±5.6a
		F. mosseae	0.28 ±0.00ab	24.13 ±0.32a	11.9 ±1.3bc	244.77 ±49.87a	120.3 ±6.9a
		C. etunicatum	0.27 ±0.00ab	22.50 ±1.32ab	10.6 ±1.4c	184.87 ±69.20a	103.5 ±6.8bc
Tomato	-P	Control	0.22 ±0.03b	22.97 ±1.55b	23.7 ±2.8a	198.50 ±14.57c	179.3 ±30.8a
		F. mosseae	0.28 ±0.02a	27.95 ±1.20a	25.5 ±2.7a	440.05 ±26.52a	191.7 ±25.0a
		C. etunicatum	0.30 ±0.01a	24.67 ±3.56ab	21.7 ±1.0a	221.03 ±27.62c	151.4 ±40.1a
	+P	Control	0.29 ±0.01a	20.85 ±0.21b	20.7 ±1.8a	254.00 ±53.03c	163.3 ±14.5a
		F. mosseae	0.30 ±0.02a	20.73 ±1.31b	26.3 ±7.9a	355.00 ±44.55b	155.4 ±18.0a
		C. etunicatum	0.30 ±0.02a	22.13 ±2.10b	20.3 ±2.3a	238.53 ±30.66c	195.7 ±36.0a
Eggplant	-P	Control	0.27 ±0.05a	20.93 ±1.10b	13.9 ±0.3c	200.45 ±51.27ab	121.1 ±2.8b
		F. mosseae	0.31 ±0.02a	25.00 ±3.82a	18.9 ±2.6bc	190.90 ±14.28b	141.0 ±2.4a
		C. etunicatum	0.31 ±0.01a	21.47 ±1.47b	31.6 ±6.7a	247.70 ±42.57ab	137.0 ±11.8ab
	+P	Control	0.31 ±0.01a	24.17 ±0.29ab	29.3 ±4.5ab	287.40 ±20.22a	146.1 ±5.3a
		F. mosseae	0.30 ±0.01a	21.77 ±1.69b	26.5 ±5.0ab	252.15 ±54.52ab	134.2 ±7.6ab
		C. etunicatum	0.30 ±0.01a	21.27 ±0.64b	27.1 ±6.2ab	258.05 ±11.95ab	129.8 ±0.0ab
Cucumber	-P	Control	0.23 ±0.01a	15.20 ±4.60b	11.4 ±1.7b	139.40 ±51.34a	83.2 ±9.8a
		F. mosseae	0.22 ±0.01a	32.72 ±2.81a	12.1 ±2.1ab	160.45 ±8.03a	92.6 ±13.1a
		C. etunicatum	0.22 ±0.02a	14.98 ±2.79b	16.5 ±2.6a	135.15 ±47.83a	81.7 ±12.1a
	+P	Control	0.23 ±0.00a	25.94 ±4.72a	13.6 ±3.5ab	145.57 ±30.31a	96.1 ±6.6a
		F. mosseae	0.22 ±0.02a	28.32 ±3.20a	14.2 ±0.3ab	179.17 ±5.94a	91.3 ±15.5a
		C. etunicatum	0.22 ±0.01a	33.92 ±4.19a	14.3 ±2.5ab	106.89 ±3.23a	89.7 ±9.2a

TABLE 3. Nutrient concentration in plant leaves.

Mean of three replicates \pm standard deviation. Means followed by different letters within the same column indicate significant differences (p < 0.05). F: *Funneliformis*, C: *Claroideoglomus*.

Plant	Sources	DF	Yield	Infection	Р	F۵	Cu	Zn	Mn
Danaan		4	0.005	0.2000	0.0470	0.0040	0.0007	0.0040	0.0440
Pepper	Р	1	0.005	0.3098	0.8470	0.3312	0.0327	0.0016	0.0418
	Mycorrhizae	2	0.022	0.0001	0.0068	0.2525	0.1089	0.0443	0.0179
	P × Mycorrhizae	2	0.090	0.9334	0.1432	0.6051	<0.0001	0.3449	0.0180
Tomato	Р	1	0.890	0.0642	0.0184	0.9596	0.3516	0.0068	0.9790
	Mycorrhizae	2	0.161	<0.0001	0.0081	0.0001	0.1438	0.2597	0.9716
	P × Mycorrhizae	2	0.213	0.4991	0.0106	0.0442	0.6924	0.1557	0.1842
Eggplant	Р	1	0.366	0.0288	0.6311	0.0477	0.0660	0.7533	0.3521
	Mycorrhizae	2	0.625	<0.0001	0.4655	0.5054	0.1156	0.2392	0.5968
	P × Mycorrhizae	2	0.975	0.5306	0.1754	0.3871	0.0650	0.0218	0.0172
Cucumber	Р	1	0.755	0.4987	0.6980	0.9526	0.3238	0.0086	0.2317
	Mycorrhizae	2	0.271	<0.0001	0.2030	0.1704	0.1453	0.0275	0.7126
	P × Mycorrhizae	2	0.713	0.6185	0.9349	0.5804	0.2587	0.0133	0.6094

DF: Degree of freedom. P indicates significant differences (p < 0.05).



Discussion

Mycorrhizal colonization

Root colonization was observed as 51-80% in +Mycorrhizae plants while maximum 20% (Figure 1) was observed in -Mycorrhizae plants which could be due to non-sterilization of soil and indigenous presence of AM fungal species, moreover, contamination with several other factors in the field for -Mycorrhizae plants (Eftekhari et al., 2012). Moderate soil P availability in the experimental treatments of pepper, tomato, eggplant and cucumber did not significantly inhibit root colonization (Cely et al., 2016). The insignificant variation in root infection of +P × Mycorrhizae in all plants further endorsed that moderate phosphate availability can allow mycorrhizal colonization, promoting plant growth (Taffouo et al., 2014). The +P significantly controlled mycorrhizal colonization. Soil P supply which exceeds P requirements of the crop may preclude mycorrhizal development (Grant et al., 2005) as observed in the +P × Mycorrhizae treatments of pepper, tomato, eggplant and cucumber.

Plant yield

Yield increase percentage was significant in -P treatments. In this study, AM fungi application acted as bio-stimulant for plant growth and increased yield by 8-83%, depending upon AM fungus species used against plant species. The stimulation of root auxin production after mycorrhizal inoculation may explain increase of root growth observed in mycorrhizae-inoculated plants (Ludwig-Müller and Güther, 2007). Yield was enhanced for pepper and tomato by 115% and 68%, respectively, due to +Mycorrhizae (Colla et al., 2015). The +Mycorrhizae plants always grow better than control or -Mycorrhizae plants (Ortaș, 2010). Effect of C. etunicatum and Gigaspora margarita on eggplant growth was evaluated, and significant improvement was found in growth parameters of +Mycorrhizae plants than -Mycorrhizae (Matsubara et al., 1995). A positive linear relationship between yield increase and +P was observed on +Mycorrhizae plants (Mahanta et al., 2014). Phosphorus increases the number of blossoms during early growth and early fruit set, thus, increases tomato fruit yield (Sainju et al., 2003).

This study found that cucumber plants were highly mycorrhizal dependent with 79.5% and 79% for both -P × *C. etunicatum* and +P × *C. etunicatum*. Mycorrhizal dependency is found high in plants under -P soil than +P soil typical of highly productive agricultural soils (Gemma et al., 2002). Since plant yield is highly dependent on nutrients availability at different growth stages and particularly fruit formation, mycorrhizal inoculation could be significant contributor for optimum nutrient uptake and plant growth. Availability of P in soil determined the IE and soil with -P resulted high IE (Plenchette et al., 1983) which assists in P_2O_5 uptake by plant roots. In contrary, in +P × Mycorrhizae, its uptake was significantly indifferent than -P × Mycorrhizae. Besides that, +/-P × Mycorrhizae treated plants showed more P-uptake than -/+P × control in all the studied plants (Bücking and Shachar-Hill, 2005).

Plant nutrient concentration

Availability of soil P_2O_5 is a key factor for plant growth and AM fungi regulation which is also dependent upon nature of mycorrhization as found in different combinations of -/+P with *F. mosseae* and *C. etunicatum* against various plants in field study (Gutjahr and Parniske, 2013). In the -P soil, AM fungal activity was high and enhanced leaf P concentration in pepper and eggplant which could be due to presence of actinomycetes in rhizosphere of +Mycorrhizae plants (Mechri et al., 2014). As it is observed that actinomycetes presence biochemically accelerates siderophores release in the rhizosphere to make complex with P adsorbents in P solubilization enhancement, P availability for the mycorrhizae and plant increases (Hamdali et al., 2008a). Actinomycetes stimulate P concentration of plant tissues in contrary to non-treated plants (Hamdali et al., 2008b). Besides that, high level of trehalose in +Mycorrhizae plants could be the reason of high intracellular P (Pi) concentration which remobilize polyphosphates (Bücking and Heyser, 2003).

Under field conditions, $-P \times F$. mosseae plants improved P uptake in pepper and tomato whereas Zn nutrition was improved in all plants with exception of pepper (Thompson et al., 2013). In this study, Zn concentration was observed high for $-P \times$ Mycorrhizae plants in comparison to -Mycorrhizae plants. Similarly, 25% increase in Zn uptake of +Mycorrhizae plants was observed by Marschner and Dell (1994). Mechanism of Zn uptake by +Mycorrhizae plants and its metabolization is not well revealed yet, but it is supposed that presence of glomalin can enhance Zn availability which forms sticky string bag-hyphae for metallic cations adsorption (Rillig et al., 2001). Another possible reason could be the improved Zn fractionation pattern in soil which assists in availability for the +Mycorrhizae plants (Subramanian et al., 2009).

In our investigation, Cu concentration in leaf was significantly influenced by +P in pepper while +Mycorrhizae also significantly enhanced Cu concentration in pepper. For Fe uptake, eggplant exhibited significant results for +P treatment. But for the Mn concentration in leaves, -/+P × Mycorrhizae had different concentration than respective control. Similarly, mycorrhizal application enhanced Mn uptake as also observed by Taylor and Harrier (2001). In rhizosphere, there are some Mn reducers which increase availability to the plants (Marschner and Timonen, 2006). In another study for olive plants (Lemanceau et al., 2009), Fe availability and transportation was enhanced by +Mycorrhizae. Possible reason for Fe availability could be the chelation due to AM fungi and rhizobacteria in the rhizosphere. Changes in microbial communities rather than the increase of microbial biomass in soil can contribute to enhanced Fe accumulation in plants (de Santiago et al., 2013).

Conclusion

Success of AM fungi application depends upon a number of factors, such as plant variety/species, environmental conditions, AM fungi species and growth parameters observed. Compatibility of AM fungi with plant species being a key factor of growth is discussed in the study by observing various parameters such as inoculation effectiveness, nutrient concentration and yield enhancement under field conditions with two levels of P_2O_5 application. Plant species are highly dependent on mycorrhizal inoculation, and IE was significant with low level of P application. Results showed that, -P × *E. mosseae* and -P × *C. etunicatum* significantly increased pepper and tomato yield, respectively. This study recommends the use of AM fungi for certain horticultural crops by considering plant growth enhancement and quality, particularly in regions of Mediterranean climate.



References

Benton, J.J. (1998). Plant Nutrition: Manual (CRC Press). https://doi. org/10.1201/9781420049190.ch4.

Bücking, H., and Heyser, W. (2003). Uptake and transfer of nutrients in ectomycorrhizal associations: interactions between photosynthesis and phosphate nutrition. Mycorrhiza *13*, 59–68. https://doi.org/10.1007/s00572-002-0196-3.

Bücking, H., and Shachar-Hill, Y. (2005). Phosphate uptake, transport and transfer by the arbuscular mycorrhizal fungus *Glomus intraradices* is stimulated by increased carbohydrate availability. New Phytol. *165*, 899–912. https://doi.org/10.1111/j.1469-8137.2004.01274.x.

Cely, M.V.T., De Oliveira, A.G., de Freitas, V.F., de Luca, M.B., Barazetti, A.R., Santos, I.M.O., Gionco, B., Garcia, G.V., Prete, C.E.C., and Andrade, G. (2016). Inoculant of arbuscular mycorrhizal fungi (*Rhizophagus clarus*) increase yield of soybean and cotton under field conditions. Front. Micro. 7. https://doi.org/10.3389/fmicb.2016.00720.

Chapman, H.D., and Pratt, P.F. (1962). Methods of analysis for soils, plants and waters. Soil Sci. *93*, 68. https://doi. org/10.1097/00010694-196201000-00015.

Chen, K., Liu, W.X., Guo, S.X., Liu, R.J., and Li, M. (2012). Diversity of arbuscular mycorrhizal fungi in continuous cropping soils used for pepper production. Afric. J. Micro. Res. *6*, 2469–2474.

Colla, G., Rouphael, Y., Di Mattia, E., El-Nakhel, C., and Cardarelli, M. (2015). Co-inoculation of *Glomus intraradices* and *Trichoderma atroviride* acts as a biostimulant to promote growth, yield and nutrient uptake of vegetable crops. J. Sci. Food Agri. *95*, 1706–1715. https://doi.org/10.1002/jsfa.6875.

De Santiago, A., García-López, A.M., Quintero, J.M., Avilés, M., and Delgado, A. (2013). Effect of *Trichoderma asperellum* strain T34 and glucose addition on iron nutrition in cucumber grown on calcareous soils. Soil Biol. Biochem. *57*, 598–605. https://doi.org/10.1016/j. soilbio.2012.06.020.

Dorais, M., Ehret, D.L., and Papadopoulos, A.P. (2008). Tomato (*Solanum lycopersicum*) health components: from the seed to the consumer. Phytochem. Rev. 7, 231–250. https://doi.org/10.1007/s11101-007-9085-x.

Eftekhari, M., Alizadeh, M., and Ebrahimi, P. (2012). Evaluation of the total phenolics and quercetin content of foliage in mycorrhizal grape (*Vitis vinifera* L.) varieties and effect of postharvest drying on quercetin yield. Ind. Crop. Prod. *38*, 160–165. https://doi. org/10.1016/j.indcrop.2012.01.022.

Gemma, J.N., Koske, R.E., and Habte, M. (2002). Mycorrhizal dependency of some endemic and endangered Hawaiian plant species. Am. J. Bot. *89*, 337–345. https://doi.org/10.3732/ajb.89.2.337.

Gioannetti, M., and Mosse, B. (1980). An evaluation of techniques for measuring vesicular-arbuscular mycorrhiza in roots. New Phytol. *84*, 489–500. https://doi.org/10.1111/j.1469-8137.1980.tb04556.x.

Grant, C., Bittman, S., Montreal, M., Plenchette, C., and Morel, C. (2005). Soil and fertilizer phosphorus: Effects on plant P supply and mycorrhizal development. Can. J. Plant Sci. *85*, 3–14. https://doi. org/10.4141/P03-182.

Gutjahr, C., and Parniske, M. (2013). Cell and developmental biology of arbuscular mycorrhiza symbiosis. Ann. Rev. Cell and Developm. Biol. *29*(1), 593–617.

Hamdali, H., Bouizgarne, B., Hafidi, M., Lebrihi, A., Virolle, M.J., and Ouhdouch, Y. (2008a). Screening for rock phosphate solubilizing actinomycetes from Moroccan phosphate mines. Appl. Soil Ecol. *38*, 12–19. https://doi.org/10.1016/j.apsoil.2007.08.007.

Hamdali, H., Hafidi, M., Virolle, M.J., and Ouhdouch, Y. (2008b). Growth promotion and protection against damping-off of wheat by two rock phosphate solubilizing actinomycetes in a P-deficient soil under greenhouse conditions. Appl. Soil Ecol. *40*, 510–517. https://doi.org/10.1016/j.apsoil.2008.08.001.

Hart, M., Ehret, D.L., Krumbein, A., Leung, C., Murch, S., Turi, C., and Franken, P. (2015). Inoculation with arbuscular mycorrhizal fungi improves the nutritional value of tomatoes. Mycorrhiza *25*, 359–376. https://doi.org/10.1007/s00572-014-0617-0.

Khalil, S., Loynachan, T.E., and Tabatabai, M.A. (1999). Plant determinants of mycorrhizal dependency in soybean. Agron. J. *91*, 135–141. https://doi.org/10.2134/agronj1999.000219620091000 10021x.

Kormanik, P.P., Bryan, W.C., and Schultz, R.C. (1977). Influence of endomycorrhizae on growth of sweetgum seedlings from eight mother trees. For. Sci. *23*, 500–505.

Kosola, K.R., Workmaster, B.A.A., and Spada, P.A. (2007). Inoculation of cranberry (*Vaccinium macrocarpon*) with the ericoid mycorrhizal fungus *Rhizoscyphus ericae* increases nitrate influx. New Phyt. *176*, 184–196. https://doi.org/10.1111/j.1469-8137.2007.02149.x.

Kowalska, I., Konieczny, A., Gastol, M., Sady, W., and Hanus-Fajerska, E. (2015). Effect of mycorrhiza and phosphorus content in nutrient solution on the yield and nutritional status of tomato plants grown on rockwool or coconut coir. Agri. Food Sci. *24*, 39–51.

Latef, A., and He, C.X. (2014). Does inoculation with *Glomus mosseae* improve salt tolerance in pepper plants? J. Plant Grow. Reg. *33*, 644–653. https://doi.org/10.1007/s00344-014-9414-4.

Lemanceau, P., Bauer, P., Kraemer, S., and Briat, J.-F. (2009). Iron dynamics in the rhizosphere as a case study for analyzing interactions between soils, plants and microbes. Plant Soil *321*, 513–535. https://doi.org/10.1007/s11104-009-0039-5.

Ludwig-Müller, J., and Güther, M. (2007). Auxins as signals in arbuscular mycorrhiza formation. Plant Sign. Behav. 2, 194–196. https://doi.org/10.4161/psb.2.3.4152.

Mahanta, D., Rai, R.K., Mishra, S.D., Raja, A., Purakayastha, T.J., and Varghese, E. (2014). Influence of phosphorus and biofertilizers on soybean and wheat root growth and properties. Field Crops Res. *166*, 1–9. https://doi.org/10.1016/j.fcr.2014.06.016.

Marschner, H., and Dell, B. (1994). Nutrient uptake in mycorrhizal symbiosis. Plant Soil *159*, 89–102. https://doi.org/10.1007/BF00000098.

Marschner, P., and Timonen, S. (2006). Bacterial community composition and activity in rhizosphere of roots colonized by arbuscular mycorrhizal fungi. In Microbial Activity in the Rhizoshere, K.G. Mukerji, C. Manoharachary, and J. Singh, eds. (Berlin, Heidelberg: Springer), pp. 139–154. https://doi.org/10.1007/3-540-29420-1_8.

Matsubara, Y., Tamura, H., and Harada, T. (1995). Growth enhancement and verticillium wilt control by vesicular-arbuscular mycorrhizal fungus inoculation in eggplant. J. Jap. Soc. Hortic. Sci. *64*, 555–561. https://doi.org/10.2503/jjshs.64.555.

Mechri, B., Manga, A.G.B., Tekaya, M., Attia, F., Cheheb, H., Meriem, F.B., Braham, M., Boujnah, D., and Hammami, M. (2014). Changes in microbial communities and carbohydrate profiles induced by the mycorrhizal fungus (*Glomus intraradices*) in rhizosphere of olive trees (*Olea europaea* L.). Appl. Soil Ecol. *75*, 124–133. https://doi. org/10.1016/j.apsoil.2013.11.001.

Ortas, I. (2010). Effect of mycorrhiza application on plant growth and nutrient uptake in cucumber production under field conditions. Span. J. Agri. Res. *8*, S116–S122. https://doi.org/10.5424/sjar/201008S1-1230.



Ortas, I., Feldmann, F., Kapulnik, Y., and Baar, J. (2008). Field trials on mycorrhizal inoculation in the Eastern Mediterranean horticultural region. Paper presented at: Mycorrhiza Works: Proceedings of the International Symposium "Mycorrhiza for Plant Vitality" and the Joint Meeting for Working Groups 1-4 COST Action 870, Hannover, Germany, 3–5 October, 2007 (DPG Selbstverlag).

Ortas, I., Sari, N., and Akpinar, C. (2003). Effect of mycorrhizal inoculation and soil fumigation on the yield and nutrient uptake of some *Solanacea* crops (tomato, eggplant and pepper) under field conditions. Agric. Mediterr. *133*, 249–258.

Pereira, J.A.P., Vieira, I.J.C., Freitas, M.S.M., Prins, C.L., Martins, M.A., and Rodrigues, R. (2016). Effects of arbuscular mycorrhizal fungi on *Capsicum* spp. J. Agri. Sci. *154*, 828–849. https://doi.org/10.1017/S0021859615000714.

Plenchette, C., Fortin, J., and Furlan, V. (1983). Growth responses of several plant species to mycorrhizae in a soil of moderate P-fertility. Plant Soil *70*, 199–209. https://doi.org/10.1007/BF02374780.

Rillig, M.C., Wright, S.F., Nichols, K.A., Schmidt, W.F., and Torn, M.S. (2001). Large contribution of arbuscular mycorrhizal fungi to soil carbon pools in tropical forest soils. Plant Soil 233, 167–177. https://doi.org/10.1023/A:1010364221169.

Sainju, U.M., Dris, R., and Singh, B. (2003). Mineral nutrition of tomato. Food Agri. Environ. 1, 176–183.

Schüßler, A., and Walker, C. (2010). The *Glomeromycota*: a species list with new families and new genera. (Kew: The Royal Botanic Garden; Munich: Botanische Staatssammlung; and Corvallis OR: Oregon State University).

Subramanian, K.S., Tenshia, V., Jayalakshmi, K., and Ramachandran, V. (2009). Biochemical changes and zinc fractions in arbuscular mycorrhizal fungus (*Glomus intraradices*) inoculated and uninoculated soils under differential zinc fertilization. Appl. Soil Ecol. *43*, 32–39. https://doi.org/10.1016/j.apsoil.2009.05.009.

Taffouo, V.D., Ngwene, B., Akoa, A., and Franken, P. (2014). Influence of phosphorus application and arbuscular mycorrhizal inoculation on growth, foliar nitrogen mobilization, and phosphorus partitioning in cowpea plants. Mycorrhiza *24*, 361–368. https://doi.org/10.1007/s00572-013-0544-5.

Tawaraya, K., Tokairin, K., and Wagatsuma, T. (2001). Dependence of *Allium fistulosum* cultivars on the arbuscular mycorrhizal fungus, *Glomus fasciculatum*. Appl. Soil Ecol. *17*, 119–124. https://doi. org/10.1016/S0929-1393(01)00126-3.

Taylor, J., and Harrier, L.A. (2001). A comparison of development and mineral nutrition of micropropagated *Fragaria* ×*ananassa* cv. Elvira (strawberry) when colonised by nine species of arbuscular mycorrhizal fungi. Appl. Soil Ecol. *18*, 205–215. https://doi. org/10.1016/S0929-1393(01)00164-0.

Thompson, J.P., Clewett, T.G., and Fiske, M.L. (2013). Field inoculation with arbuscular-mycorrhizal fungi overcomes phosphorus and zinc deficiencies of linseed (*Linum usitatissimum*) in a vertisol subject to long-fallow disorder. Plant Soil *371*, 117–137. https://doi. org/10.1007/s11104-013-1679-z.

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Addresses of authors:

- M. Rafique^{1,2} and İ. Ortas^{1,*}
- ¹Department of Soil Science and Plant Nutrition, University of Çukurova, Faculty of Agriculture, Adana, Turkey
- ² Department of Plant Sciences, Quaid-I-Azam University, Islamabad, Pakistan
- * Corresponding author; E-mail: iortas@cu.edu.tr

