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Mycorrhizae enhances horticultural plant yield and nutrient uptake under phosphorus deficient field soil condition

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ABSTRACT

Mycorrhizae can enhance plant growth and phosphorus (P) use efficiency in horticultural plants. This research evaluated the effectiveness of mycorrhizae on increasing growth and yield of nine horticultural plants for two different rates of P fertilization under field conditions. The mycorrhizal inoculums increased the root colonization of mycorrhizal horticultural plants compared with the non-inoculated treatments. Mycorrhizal inoculation significantly increased onion, garlic, chickpea, broad bean, carrot, parsley and cress plants. Mycorrhizal effectiveness showed that without P addition the effect of mycorrhizae on plant yield is much higher than that with P fertilizer addition. Under low P fertilization inoculation effectiveness is much higher than P addition treatments. Mycorrhizal inoculums also increased tissue P and zinc (Zn) for horticultural plant. The improved growth, yield, Zn and P uptake in plants demonstrated the potential of mycorrhizal inoculation to reduce the effects P fertilization on horticultural plants grown under field conditions.

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KEYWORDS

Glomus mossae; inoculation effectiveness; mycorrhizal vegetable plants; synergistic interaction; Zn and P uptake

Introduction

Arbuscular Mycorrhizae Fungi (AMF) lives in symbiosis with the majority of plant species. These fungi increase the absorption of surface roots, resulting in an increased uptake of nutrients, especially less mobile nutrients including zinc (Zn) and copper (Cu) by plants (Smith and Read 1997; Li et al. 1991; Marschner 1996; Ortas 2012). The effect of AMF on Zn uptake by plants may depend on the quantity of both internal and external fungal hyphae (Christie et al. 2004). Many environmental factors have an influence on the development of mycorrhizal structures (Smith and Read 1997). One of the factors, which reduces the growth of AMF is high concentrations of phosphorus (P) in the plant root zone (Kowalska et al. 2015). Despite the weaker development of mycorrhizal structures in the plants grown at the optimal P concentration, AMF decreased Zn content within plant tissue (Konieczny and Kowalska 2017). In contrast, AMF can able to increase Zn uptake by exchange with plant carbohydrate (Cavagnaro 2008). Investigation on mechanisms involved stimulation of Zn uptake by mycorrhizal plants in a situation of P deficient or efficient soil under field conditions is still required.

Mycorrhizae have biochemical and physiological characteristics which differ from those of roots that can enhance P availability from soil (Cynthia et al. 2004). Mycorrhizal plants can absorb more P at lower concentration in the soil solution than non-mycorrhizal plants (Plenchette and Morel 1996). Hence, soil P has direct effect on AM external hyphal growth

(Miranda and Harris 1994). The AM colonization of plants and mycorrhizal infectivity are decreased by both mineral and organic P fertilizers (Ortas 2003; Lakshmipathy et al. 2007). Thus, the AM symbioses are critical to P fertilization and that mycorrhizal benefits are usually inversely related to availability of P (Grant et al. 2005).

The AM symbiosis increases crop yields (Harrier and Watson 2003). In general, there were variations in response to colonization of mycorrhizae species occur among different plant species due to their abiotic and biotic factors (Ronsheim 2012). Mycorrhizal inoculation usually increases the growth of tomato, pepper and eggplant (Ortas 2003), watermelon, capsicum, cucumber (Ortas 2012; Ortas 2010) especially under conditions of low P availability. Similarly, a study found that *S. viscosum* plant increased the biomass production compared to non-mycorrhizal plants in a field soil that has low P concentration (Waed et al. 2017). A survey of 78 published field trials found that increased AMF colonization resulted in an average yield increase of 37% percent (McGonigle 1988). Another study of 290 published field and greenhouse studies determined that increased colonization resulted in a 23% yield increase (Lekberg and Koide 2005). However, the effect of the different mycorrhizal horticultural plant species growth responses of the various plant part productivity under low and high field soil P availability condition is still unknown. Many experiments were conducted under greenhouse pot conditions; still there is a need to use mycorrhizae under field conditions. Also, more details have yet to be indicated on the interactions between AMF and P fertilization for several horticultural plant species under field conditions (Hagh et al. 2016). Quantitative analysis of the mechanisms employed by mycorrhizal associations in P fertilization for horticultural plants is still required to understand the contribution of these associations in the functioning of the soil-plant-root systems. To be relevant for agronomic purposes, this analysis should be developed under field conditions representative of commercial production systems. Horticultural crop species are extensively grown under the Mediterranean climate conditions. Soil under this climate has high clay content, high soil pH and low organic carbon. Usually soil fertility is low and nutrient availability especially P and Zn absorption is limited. Mycorrhiza inoculation may be ecological solution for sustainable agricultural production. Our aim was to address three questions: (i) How do AMF affect horticultural plant productivity under P deficient and efficient condition? (ii) How AMF and P regulates P and Zn uptake by horticultural plant species? (iii) Do mycorrhizae inoculation affect horticultural plants growth and yield under field conditions? We hypothesized that (i) mycorrhizal inoculation reduces P fertilizer requirement for horticultural vegetable plants (ii) mycorrhizal inoculation increase nutrient (Zn and P) uptake and productivity of horticultural plant and (iii) P fertilization reduces inoculation effectiveness.

Materials and methods

Experimental site

The experimental site was located at the Research Farm of the Cukurova University (37°00'54.31"N, and 35°21'21.56 "E and 31 m above mean sea level) in eastern part of the Mediterranean region, Adana, Turkey. The annual precipitation of the experimental site was 670.8 mm of which about 80% precipitation is received between November and April with a mean annual humidity of 66% (Ortas 2012; Anonymous 2008).

Experimental soil

Basic physiochemical properties of this experimental soil are shown in Table 1. Soil physiochemical properties were analyzed (Page et al. 1982). Indigenous spores were extracted from the field soil samples taken in early autumn using the wet-sieving technique (Jones 1998).

Table 1. Experimental field soil physical, chemical and biological properties.

Soil pH (in H ₂ O)	OC	IOC	TN	Clay	Silt	Sand	CEC	P	Fe	Mn	Zn	Cu	No. of AMF spores in 10 g soil
	g kg ⁻¹ soil			g kg ⁻¹			cmol + kg ⁻¹	mg kg ⁻¹					
7.56	0.96	3.77	0.08	318.8	360.9	320.3	20.50	14.50	2.48	3.84	0.24	1.16	98

All values were means of three replicates.

Experimental design and treatments

The experiment was conducted in a randomized complete block design (RCBD) with three replications. In each block, phosphorus (P) application was main treatment as -P (0 kg P₂O₅) and +P (100 kg P₂O₅) with and without mycorrhizal inoculation. The *Glomus mosseae* was used for the mycorrhizae species.

Field experimental procedure

Several crops such as onion (*Allium cepa* L.), garlic (*Allium sativum* L.), radish (*Raphanus raphanistrum* subsp. Sativas), carrot (*Daucus carota* subsp. Sativus), parsley (*Petroselinum crispum* L.), roka (*Eruca sativa* L.), chickpea (*Cicer arietinum* L.), broad bean (*Vicia faba* L.), and cress (*Lepidium sativum* L.) were used as experimental plants. The varieties used for onion, garlic, radish, carrot, parsley, roka, chickpea were Kantaropu, Kastamonu, Havuç, Opolanka, flat leaf, arugula, kabuli Seeds were sown in the field and mycorrhizal spores (*G. mosseae*) were calculated based on the number of spores present in 10 g inoculum (mix of spores, hyphae, root and growth medium) under 1000 spores which were placed approximately 50 mm below the seeds. In non-mycorrhizal treatments, the same amount of mycorrhizae free substrate (growth medium) was used. Each plant species was the subject of a separate experiment according to their ecological growth period. Since all plants species are grown during winter time they were seeded in the late autumn. The plots sizes for all crops were 2 × 5 = 10 m². A complete randomized block design with three replications was used with each block containing two treatments (mycorrhizal and non-mycorrhizal plots). Plant yield and fruits were harvested several times depending on plant species and fruit production/ripeness. Parsley and roka were harvested 6 times. Broad bean was harvested 3 times and rest of the plants were harvested at maturity levels.

Morphological analysis of roots

The roots were separated from the shoots. The separated roots were washed 3-4 times with deionized water. Then, the whole root system was scanned by a root-system scanner. Root length was measured using the software WinRHIZO image analysis system (WIN MAC, Regent Instruments Inc., Quebec, Canada, <http://www.regentinstruments.com/>) (Arsenault et al. 1995). The scanner was set according to several plants for standardization of the scanner area.

Leaf P and Zn analysis

Before each crop flowered upper mature plant leaves were taken for nutrient analysis. Plants leaves were taken.^[25] Plant leaves 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% hydrochloric acid (HCl). Leaf P concentration was determined with the vanadate--molybdate yellow colorimetric method using a spectrophotometer and Zn concentration was determined by inductively coupled plasma (ICP) (Chapman and Pratt 1961).

Mycorrhizal colonization

Before plant flowering, the roots of the two plants were carefully removed and washed for mycorrhizal colonization assessment. The root clearing and staining procedure were followed (Koske and Gemma 1989). The percentage of AMF colonization was calculated as the number of 10-mm-long root segments identified as colonized under a stereo microscope at 20× magnification out of 100 root segments (Giovannetti and Mosse 1980).

Inoculation effectiveness

At harvest, fresh and dry weight of horticultural crops were recorded. Inoculation effectiveness was calculated for each crop based on the following formula:

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

where +M = mycorrhizal plant, -M = non-mycorrhizal plant

Statistical analysis

All data were statistically analyzed using the analysis of variance (ANOVA) procedure in (SAS 2009) program to assess the effects of P, mycorrhizae and mycorrhizae × P interaction. Last significance difference (LSD) at $p \geq 0.05$ was tested to determine the significant differences among treatment means.

Results

Yield of horticultural crop for mycorrhizae and P addition

Combination of mycorrhizae and P fertilizer application on yield of horticultural crops was shown in Table 2. Onion yield was highest among other horticultural crops at mycorrhizae with no P added treatment. However, no mycorrhizae effect was found when P was added. The garlic yield was maximum (10.859 kg ha⁻¹) in both P and mycorrhizae added treatment. Chickpea yield was highest only in mycorrhizae added treatment under P deficient condition. The radish yield was highest for the combined P and mycorrhizae application. The addition of P increased twice for radish plant yield as compared to control plants. However, radish yield did not differ significantly ($p \geq 0.05$) between mycorrhizae treatment. The cress plant yield was highest in P without mycorrhizae and lowest in control treatment. Combined effect of mycorrhizae and P on crop

Table 2. Effect of mycorrhizae and phosphorus fertilization on different plant yield (kg ha⁻¹) under field conditions.

Treatments		Crops yield (kg ha ⁻¹)					
Phosphorus	Mycorrhizae	Onion	Garlic	Chickpea	Broad bean	Radish	Cress
-P	-M	18229 ± 372 b	5114 ± 445 b	13 ± 3 b	4833 ± 902 b	26563 ± 4824 b	56065 ± 13935 b
	+M	34207 ± 4777 a	8288 ± 795 a	42 ± 13 a	7437 ± 1483 ab	27601 ± 5238 b	80555 ± 34777 ab
+P	-M	30763 ± 2833 a	8074 ± 2644 a	29 ± 2 a	9437 ± 2207 a	41583 ± 3408 a	108680 ± 8541 b
	+M	31748 ± 10199 a	10859 ± 97 a	38 ± 8 a	9451 ± 1385 a	48566 ± 7971 a	87853 ± 5865 ab
<i>Analysis of variance</i>		<i>p- value</i>					
Phosphorus		n.s.	*	n.s.	**	***	*
Mycorrhizae		*	**	**	n.s.	n.s.	n.s.
Phosphorus × mycorrhizae		n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Different letters indicate significant differences ($p < 0.05$). The lower part of the table shows p -values from the analysis of variance. n.s., *, ** and *** represent probability of > 0.05 , ≤ 0.05 , ≤ 0.01 and ≤ 0.001 . Values were means of three replicates ± standard deviation. -M no AMF inoculums, +M with AMF inoculums used, -P = 0 kg P2O5, +P = 100 kg P2O5.

Table 3. The effect of mycorrhizae and P fertilization on different plant fresh leaves yield under field conditions kg ha⁻¹.

Treatments		Vegetables plant (kg ha ⁻¹)			
Phosphorus	Mycorrhizae	Radish	Carrot	Parsley	Roka
-P	-M	10122 ± 333 a	10277 ± 252 b	17455 ± 3605 b	8296 ± 2189b
	+M	64556 ± 1100 b	15007 ± 292 a	30015 ± 585 a	9825 ± 983b
+P	-M	10761 ± 2006 a	15547 ± 4387a	31095 ± 8775 a	17939 ± 6189a
	+M	9906 ± 206 a	15637 ± 922 a	31275 ± 1845 a	18231 ± 3964a
<i>Analysis of variance</i>			<i>p-value</i>		
Phosphorus		*	ns*	**	
Mycorrhizae		**	*n.s.	n.s.	
Phosphorus × mycorrhizae		n.s.	*	n.s.	n.s.

Different letters indicate significant differences ($p < 0.05$). The lower part of the table shows p -values from the analysis of variance. n.s., *, ** and *** represent probability of > 0.05 , ≤ 0.05 , ≤ 0.01 and ≤ 0.001 . Values were means of three replicates ± standard deviation. -M no AMF inoculums, + with AMF inoculums used, -P = 0 kg P2O5, +P = 100 kg P2O5.

productivity was found for garlic, broad bean and radish plant. Only mycorrhizae effect on crop productivity was found for onion and chickpea. Likewise, P effect on crop productivity was found for cress plant.

Effect of mycorrhizal inoculation and P fertilization on plant fresh yield

The effect of mycorrhizae and P on plant fresh leaves is shown in Table 3. When yield is expressed as fresh leaves, such as parsley and roka, productivity was affected by both mycorrhizae and P application to the field. Interestingly, only mycorrhizae had positives effect on radish plant fresh leaf that increased six times higher as compared to other amendments. Similarly, radish plants provided highest fresh leaf yield in mycorrhizae added plot. However, fresh leaf weight of radish plant did not differ significantly ($p \geq 0.05$) with and without P application. But, radish leaf weight declined significantly ($p \geq 0.05$) when P was added with mycorrhizae. In general, parsley fresh leaf significantly ($p \geq 0.05$) increased with the addition of P and mycorrhizae. Fresh leaf weight of roka plant did not differ significantly ($p \geq 0.05$) between mycorrhizae treatments. However, fresh leaf weight of roka plant increased significantly ($p \geq 0.05$) due to P application in the field.

Several horticultural plants fresh root weight varies differently irrespective to mycorrhizae and P fertilization. The carrot has both mycorrhizae and P effect irrespective to fresh root weight. However, for other plant no combined effect was found. No significant P effect was found for the root weight of parsley and roka plant. But, only P effect was found for cress plant fresh root weight. The effect of mycorrhizae and P fertilization on different plant fresh root yield is shown in Table 4. The mycorrhizae addition significantly ($p \geq 0.05$) decreased the root weight of radish and roka plant. The highest fresh carrot root was found in combined mycorrhizae and P treatment. The parsley fresh root weight did not differ significantly ($p \geq 0.05$) among all treatments. The fresh root yield of roka plant reduced significantly ($p \geq 0.05$) due to mycorrhizae amendment. Likewise, the fresh root yield of cress plant increased 1.5 times due to mycorrhizae addition for the without P added treatment. In contrast, fresh root yield of cress plant reduced to half due to mycorrhizae addition on with P added treatment.

Effect of mycorrhizae and P on root colonization

The root colonization was significantly affected by the mycorrhizal inoculation. The extent of arbuscular mycorrhizal colonization differed among several horticultural plants. The root colonization results showed that parsley plant had highest root colonization followed by carrot, onion, chickpea, broad bean, garlic and cress plant. However, no root colonization was found for radish and roka plant (Table 5).

Table 4. Effect of mycorrhizae and P fertilization on different plant fresh root yield (kg ha⁻¹) under field conditions.

Treatments		Fresh root yield (kg ha ⁻¹)				
Phosphorus	Mycorrhizae	Radish	Carrot	Parsley	Roka	Cress
-P	-M	12600 ± 6089 a	23930 ± 770 b	4233 ± 183 a	15608 ± 5718 a	7332 ± 1190 b
	+M	3361 ± 1017 b	47520 ± 5060 a	4764 ± 17 a	6934 ± 15 b	10601 ± 2066 ab
+P	-M	7083 ± 1994 ab	42580 ± 3500 a	5826 ± 1841 a	16429 ± 4986 a	12352 ± 2759 a
	+M	4889 ± 133 b	49760 ± 8000 a	4845 ± 98 a	14149 ± 3158 ab	7178 ± 188 b
<i>Analysis of variance</i>				<i>p-value</i>		
Phosphorus		n.s.	**	n.s.	***	
Mycorrhizae		*	***	n.s.	n.s.	n.s.
n.s. Phosphorus × mycorrhizae		n.s.	*	n.s.	n.s.***	ns

Different letters indicate significant differences ($p < 0.05$). The lower part of the table shows p -values from the analysis of variance. n.s., *, ** and *** represent probability of > 0.05 , ≤ 0.05 , ≤ 0.01 and ≤ 0.001 . Values were means of three replicates ± standard deviation. -M no AMF inoculums, + M with AMF inoculums used, -P = 0 kg P2O5, +P = 100 kg P2O5.

Table 5. Effect of mycorrhizal inoculation and P addition on different plant root colonization under field conditions.

Treatments		Root colonization of several plants (%)								
Phosphorus	Mycorrhizae	Onion	Garlic	Chickpea	Broad bean	Radish	Carrot	Parsley	Cress	Roka
-P	-M	14 ± 5 b	14 ± 7 b	13 ± 3 b	15 ± 3 a	0.0 ± 0.0 b	17 ± 0.3 c	24 ± 4 b	14 ± 6 a	0.0 ± 0.0 b
	+M	51 ± 8 a	36 ± 9 a	42 ± 13 a	39 ± 8 a	0.0 ± 0.0 b	71 ± 6 a	76 ± 11 a	18 ± 5 a	1.0 ± 1.0 ab
+P	-M	23 ± 6 b	27 ± 8 ab	29 ± 2 a	17 ± 6 a	3.0 ± 1.0 a	15 ± 2 c	17 ± 0.5 b	18 ± 4 a	0.5 ± 0.5 ab
	+M	24 ± 2 b	35 ± 6 a	38 ± 8 a	34 ± 28 a	0.0 ± 0.0 b	55 ± 4 b	67 ± 13 a	19 ± 4 a	1.5 ± 0.5a
<i>Analysis of variance</i>						<i>p-value</i>				
Phosphorus		*	n.s.	n.s.	n.s.	***	**	n.s.	n.s.	n.s.
Mycorrhizae		***	*	**	*	***	***	***	n.s.	*
Phosphorus × mycorrhizae		***	n.s.	n.s.	n.s.	***	*	n.s.	n.s.	n.s.

Different letters indicate significant differences ($p < 0.05$). The lower part of the table shows p -values from the analysis of variance. n.s., *, ** and *** represent probability of > 0.05 , ≤ 0.05 , ≤ 0.01 and ≤ 0.001 . Values were means of three replicates ± standard deviation. -M no AMF inoculums, +M with AMF inoculums used, -P = 0 kg P2O5, +P = 100 kg P2O5.

Effect of mycorrhizae and P fertilization on different plant root length

Plant root lengths varied differently irrespective to P and mycorrhizae application. The root length of radish plant did not have any effect of mycorrhizae application. But, the radish plant root length declined due to P application (Table 6). Also, mycorrhizal inoculated radish plant has less root length than non-mycorrhizal one. However, the roka plant root length significantly ($p \geq 0.05$) declined due to mycorrhizae addition with and without P fertilization condition. The root length of carrot plant significantly ($p \geq 0.05$) increased due to mycorrhizae addition and remained similar in P as well as combined P and mycorrhizae application. For parsley plant mycorrhizal inoculation and P fertilization have not any effect on root length.

Effect of mycorrhizal inoculation and P fertilization on plant tissues P and Zn concentration

In general, onion, garlic and chickpea tissue P concentration significantly ($p \geq 0.05$) increased with mycorrhizae amended soil as well as both P and mycorrhizae amended soil (Table 7). The broad bean plant tissue P concentration did not significantly differ ($p \geq 0.05$) among all treatments. The radish plant P concentration had no mycorrhizal effect, however, radish plant P concentration increased due to P fertilization. Interestingly, carrot plant had mycorrhizal effect on P concentration. The carrot plant P concentration reached 0.40% due to mycorrhizal inoculation. But, when P was added, the carrot plant tissue P concentration decreased to 0.30%. However, carrot tissue P concentration did not have any P effect and it was 0.20% with or without P addition. The parsley plant P concentration reached double when P was added to the field. The parsley plant P concentration increased 0.20% to 0.30% when mycorrhizae were added. The cress plant

Table 6. The effect of mycorrhizae and phosphorus fertilization on different plant root length under field conditions (km ha^{-1}).

Treatments		The root length of several plant (km ha^{-1})				
Phosphorus	Mycorrhizae	Radish	Carrot	Parsley	Roka	Cress
-P	-M	9000 ± 4224 a	223 ± 21 b	524 ± 23 a	103 ± 11 ab	616 ± 244 ab
	+M	2025 ± 205 b	428 ± 45 a	575 ± 85 a	36 ± 9 c	619 ± 131 ab
+P	-M	5713 ± 2949 ab	448 ± 72 a	518 ± 146 a	115 ± 49 a	915 ± 332 a
	+M	2077 ± 1738 b	383 ± 31 a	627 ± 6 a	61 ± 23 cb	431 ± 45 b
<i>Analysis of variance</i>				<i>p-value</i>		
Phosphorus		n.s.	**	n.s.	n.s.	n.s.
Mycorrhizae		**	*	n.s.	**	n.s.
Phosphorus × mycorrhizae		n.s.	***	n.s.	n.s.	n.s.

Different letters indicate significant differences ($p < 0.05$). The lower part of the table shows p -values from the analysis of variance. n.s., *, ** and *** represent probability of > 0.05 , ≤ 0.05 , ≤ 0.01 and ≤ 0.001 . Values were means of three replicates ± standard deviation. -M no AMF inoculums, +M with AMF inoculums used, -P = 0 kg P2O5, +P = 100 kg P2O5.

Table 7. Effect of mycorrhizal inoculation and P addition on different plant tissue P (%) under field conditions.

Treatments		Several plant tissue P (%)								
Phosphorus	Mycorrhizae	Onion	Garlic	Chickpea	Broad bean	Radish	Carrot	Parsley	Cress	Roka
-P	-M	0.2 ± 0.0 c	0.2 ± 0.0 b	0.2 ± 0.0 b	0.1 ± 0.0 a	0.2 ± 0.0 b	0.2 ± 0.0 c	0.2 ± 0.0 b	0.4 ± 0.0 b	0.3 ± 0.0 b
	+M	0.3 ± 0.0 b	0.3 ± 0.0 a	0.3 ± 0.0 a	0.2 ± 0.0 a	0.2 ± 0.0 b	0.4 ± 0.0 a	0.3 ± 0.1 ab	0.5 ± 0.1 a	0.4 ± 0.1 a
+P	-M	0.3 ± 0.0 ab	0.3 ± 0.0 a	0.3 ± 0.0 a	0.2 ± 0.1 a	0.3 ± 0.0 a	0.2 ± 0.0 c	0.4 ± 0.0 a	0.5 ± 0.0 ab	0.3 ± 0.0 ab
	+M	0.3 ± 0.0 a	0.3 ± 0.0 a	0.3 ± 0.0 a	0.2 ± 0.0 a	0.3 ± 0.0 a	0.3 ± 0.0 b	0.3 ± 0.1 ab	0.5 ± 0.0 a	0.3 ± 0.0 ab
<i>Analysis of variance</i>						<i>p-value</i>				
Phosphorus		**	*	***	n.s.	***	***	n.s.	n.s.	n.s.
Mycorrhizae		*	*	***	n.s.	n.s.	***	***	*	*
Phosphorus × mycorrhizae		n.s.	*	***	n.s.	n.s.	***	n.s.	n.s.	n.s.

Different letters indicate significant differences ($p < 0.05$, Tukey test). The lower part of the table shows p -values from the analysis of variance. n.s., *, ** and *** represent probability of > 0.05 , ≤ 0.05 , ≤ 0.01 and ≤ 0.001 . Values were means of three replicates ± standard deviation. -M no AMF inoculums, +M with AMF inoculums used, -P = 0 kg P2O5/ha, +P = 100 kg P2O5/ha.

Table 8. Effect of mycorrhizal inoculation and P addition on different plant tissue Zn (ppm) under field conditions.

Treatments		Several plant tissue Zn (mg kg^{-1})								
Phosphorus	Mycorrhizae	Onion	Garlic	Chickpea	Broad bean	Radish	Carrot	Parsley	Cress	Roka
-P	-M	18 ± 2 b	18 ± 2 b	24 ± 2 b	21 ± 1 c	34 ± 1 a	17 ± 0.5 b	19 ± 7 a	30 ± 1 b	36 ± 1 a
	+M	27 ± 5 a	24 ± 2 ab	35 ± 6 a	38 ± 8 ab	36 ± 1 a	23 ± 1 a	27 ± 3 a	25 ± 1 b	34 ± 2 ab
+P	-M	21 ± 2 ab	21 ± 5 ab	30 ± 4 ab	35 ± 7 b	27 ± 4 b	19 ± 1 b	29 ± 0.0 a	26 ± 4 b	33 ± 1 b
	+M	27 ± 3 a	25 ± 4 a	35 ± 4 a	47 ± 1 a	23 ± 1 b	23 ± 2 a	22 ± 8 a	38 ± 5 a	35 ± 0.3 ab
<i>Analysis of variance</i>						<i>p-value</i>				
Phosphorus		n.s.	n.s.	n.s.	**	***	n.s.	n.s.	*	n.s.
Mycorrhizae		**	*	*	**	n.s.	***	n.s.	n.s.	n.s.
Phosphorus × mycorrhizae		n.s.	n.s.	n.s.	n.s.	*	n.s.	n.s.	**	*

Different letters indicate significant differences ($p < 0.05$). The lower part of the table shows p -values from the analysis of variance. n.s., *, ** and *** represent probability of > 0.05 , ≤ 0.05 , ≤ 0.01 and ≤ 0.001 . Values were means of three replicates ± standard deviation. -M no AMF inoculums, +M with AMF inoculums used, -P = 0 kg P2O5/ha, +P = 100 kg P2O5/ha.

tissue P concentration has both P and mycorrhizae effect. The roka plant tissue P concentration increased significantly ($p \geq 0.05$) due to mycorrhizae effect but when P was added it did not show any effect irrespective to plant tissue P concentration.

The onion, garlic, chickpea and broad bean plant tissue Zn concentration has high mycorrhizal effect along with and without P fertilization (Table 8). The radish plant tissue Zn concentration had no mycorrhizae effect, but it had a P effect. In contrast, the carrot plant Zn concentration increased due to mycorrhizal inoculation but it had no P fertilization effect. In general, parsley plant Zn concentration did not have any mycorrhizae and P effects. The interactive effect of P and mycorrhizae on Zn concentration of cress plant was significant. However, individual P addition and mycorrhizae effect were not different irrespective of the Zn concentration for the cress

Table 9. Effect of mycorrhizal inoculation and P addition on inoculation effectiveness under field conditions.

Treatments	Inoculation effectiveness (%) for several plants								
	Onion	Garlic	Chickpea	Broad bean	Radish	Carrot	Parsley	Cress	Roka
+M-P	46.7	38.3	81.6	35	3.8	49.6	41.8	30.4	15.6
+M+P	3.1	25.6	9.4	0.2	14.4	14.4	0.6	23.7	1.6

plant. The roka plant tissue Zn concentration declined due to P fertilization however it did not have any mycorrhizae effect.

The effect of AMF inoculation on mycorrhizal effectiveness

The inoculation effectiveness of all plants studied here was higher in +M-P than +M+P treatment except radish plant (Table 9). Chickpea showed highest inoculation effectiveness among all plants. Carrot and onion have also had high inoculation efficiency but less than the chickpea. Inoculation effectiveness for radish plant was 3.8 and 14.4% for +M-P and +M+P treatments, respectively. Over all the results showed that under filed conditions without P fertilizer addition for all plant species mycorrhizae is significantly increased the plant growth except radish. However, with P addition the effect of mycorrhizae on plant growth decreased and the effect of P increased. The results significantly support the hypothesis that mycorrhizae inoculation can replace P fertilizer under field conditions.

Discussion

Effects of phosphorus fertilizer addition root colonization

Root colonization tended to be decline in several plants due to P fertilization with AMF in the field (Table 5). The P fertilization generally decreases the colonization of plant roots by AMF. This may be due to the reason that control of mycorrhizal colonization is linked to P in the host plants. The higher dry mass produced by AMF plants compared to control is due mainly to P fertilization since root colonization decreases with addition of P (Table 4). There is also the possibility that AMF combination with P availability in soil stimulates the symbiosis and maximize yield that results declined in root colonization. The onion plant root colonization reduced from 51 to 24% due to P fertilization. Similarly, carrot plant root colonization reduced from 71 to 55% for P and AMF interaction. Likewise, parsley plant root colonization declined from 76 to 67% for the P and AMF amendment in the field. A study found that maize plant root colonization reduced 28 to 12% due to 0.16 mM P fertilization on 24 days after planting (Hagh et al. 2016). Mycorrhizal fungal inoculum were introduced into 3-1 pots of Turface from established *Glomus versiforme* *Allium porrum* L. pot cultures. They concluded that reduction in colonization with increased P nutrition was a result of a reduction in development of internal colonization rather than a reduction in penetration of the root (Braunberger et al. 1991). Similarly, other study found that interactive effect of P fertilization and AMF decrease root colonization of onion by 20% (Furlan and Bernier-Cardou 1989). They examined the joined effect of P and AMF on onion production in field soil conditions. Also, they speculated that photosynthesis is translocated from host plants to spores through the connecting mycelium that results P fertilization suppresses root colonization.

Impact of P and mycorrhizal amendment on horticultural plant productivity

Mycorrhizae amendment enhances horticultural plant productivity. The onion and chickpea plant yield increased by 2–3 times for the mycorrhizae application to the field. Those findings proved one of our hypothesis that under field conditions mycorrhizal inoculation increase horticultural

plant productivity. However, the interactive effect of mycorrhizae and P did not significantly ($p \geq 0.05$) increased onion and chickpea yield (Table 2). Thus, AMF use as an alternative for increasing onion and chickpea yield because it allows decrease the dependence to inorganic chemical fertilizer like P. Other studies also concluded that the application of selected AMF as a bio-fertilizer may represent an innovative eco-sustainable practice for improving the crop profitability for growers while reducing the need for P fertilization (Conversa et al. 2013). Similarly, several field studies have shown that inoculation with efficient AMF not only increases growth and yield of crop plants but also reduces the application of P fertilizer application by nearly about 50% especially in marginal soils deficient in nutrients (Bagyaraj et al. 2015). Other field studies also estimated that a reduction of 80% of the recommended phosphorus fertilizer could be supplemented by inoculation with AM fungi (Jakobsen 1995). The results indicated that AMF have the potential to decrease dependence on P fertilizers, which are considered to be vitally important for the establishment of most crops (Novias et al. 2007) Therefore, AMF may be used to compensate for P deficiencies. These results also proved another hypothesis that mycorrhizal inoculums can reduce demand of P fertilizer for horticultural vegetable plants.

Our findings showed that carrot plant fresh leaf weight reached from 23930 to 47520 kg ha⁻¹ due to mycorrhizae addition to the field. The fresh weight of carrot plant again goes up to 49760 kg ha⁻¹ when both mycorrhizae and P were added in the field. Similarly, carrot plant fresh root yield increased 10277 to 15007 kg ha⁻¹ due to mycorrhizae supply to the field. The observed increase in the carrot plant biomass with application of AMF might be attributed in part to the inherently low AMF status of the soil used in this field experiment (Table 1). It reached again 15637 kg ha⁻¹ when both mycorrhizae and P were added in the field. This could be due to the reason that carrots were highly dependent on mycorrhizae and P for optimum growth regardless of whether the source is from AMF or applied P.^[33] However, at high or moderate levels of P, root weight of mycorrhizal carrots did not get benefit much from additional P, indicating that AMF growth enhancement is inhibited at high or moderate levels of P application to the field. Regardless of that we have found that plant tissue P uptake significantly ($p \geq 0.05$) increased on all testing plant except radish (Table 7). This increased growth of the mycorrhizal plant is mainly attributed to the enhancement of P uptake (Mohammad et al. 1998; Ortas et al. 2017)

In generally radish is not mycorrhizal, however in some work it has been indicated that under field conditions it has been shown that radish plant responded to the mycorrhizae colonization (Von Drasek 2014). In this work, radish plants have mycorrhizal colonization around <3%, which has little effect on yield increase.

The effect of AMF and P fertilizer application on inoculation effectiveness

The high inoculation effectiveness was observed when +M plants were fertilized with the -P rate under the field conditions cultivation. Findings also showed that the AMF inoculation effectiveness were significantly ($p \geq 0.05$) reduced after application of P fertilization (Table 9). This also proved our hypothesis that P fertilization declined inoculation effectiveness of mycorrhizal plant. There may be possibility that when P is scarce, plants get often benefit from AMF-plant symbiosis, and AMF increase inoculation effectiveness (Hoeksema et al. 2010)., but when P is abundant in soil, plants may receive little or no benefit from AMF, and P may therefore reduce inoculation effectiveness (Johnson 2010). This reduction in inoculation effectiveness with P fertilizer application is in agreement with the fact that P have negative effect of AMF colonization (Gryndler et al. 2005). The mechanism by which soil P reduces inoculation effectiveness is not yet clearly understood. However, several hypotheses have been proposed to explain the effects of P fertilization on mycorrhizal inoculation effectiveness. These are increased lecithin in the roots may block the mycorrhizal infection, alteration of the permeability of the root cell membranes may decrease the quantity of exudates and consequently decrease germination and mycelium growth, and root

sucrose increases may decrease root colonization because of changes in photosynthesis (Moreira and Siqueira 2006). Our speculation is that decrease in inoculation effectiveness with P fertilization indicates the beneficial effect of mycorrhizae at low P levels. The lower specific inoculation effectiveness with P and AMF may also implies a possible precipitation or/and formation of AMF-P complexes in the roots that result less inoculation effectiveness in the both mycorrhizae and P treatment. The inoculation effectiveness observed in the current study was relatively high in without P and with mycorrhizae treatment compared to with P and with mycorrhizae treatment under field condition. This could be due to the reason that differential effects of mycorrhizae are masked at higher rates of P application.

Under field conditions effect of mycorrhizal inoculation on P and Zn uptake

Mycorrhizae had a very prevalent effect on plant tissue P uptake (Tawaraya 2014). Plant tissue P uptake significantly ($p \geq 0.05$) increased in onion, garlic, chickpea, carrot, parsley, cress and roka plant due to mycorrhizae amendment in the field (Table 7). In contrast, no significant effect was found for combined P fertilization and mycorrhizae amendment on plant tissue P uptake. Similarly, other studies found that the effect of AMF on corn plant tissue P uptake increased by 35% (Shnyreva and Kulaev 1994). They speculated that P nutrition of corn plants has been shown to be an essential factor of biosynthetic processes in the plant. Root infection by AMF has greatly improved the P uptake, translocation and its subsequent transfer through the host plant. Usually AM hyphae have 2.5 to 5 times smaller diameters than plant roots and therefore have a greater surface area per unit volume. This surface area makes the fungi much more efficient than roots in the uptake of P by plants (Bolan 1991).

Our findings on AMF can make a highly significant contribution to testing plants P uptake implies that the direct P uptake pathway via root hairs and root epidermis cells must case of function in some plant/fungus combinations of the two pathways is related to plant responsiveness or AMF root colonization. Further, several root parameters such as root length, root diameter, root surface area, and root hair density are important for enhanced nutrient uptake by plants. Consequently, changes that AMF bring about on root morphology, such as root branching and root elongation, could constitute an additional mechanism by which the fungi enhance P uptake.

AMF plays an important role in Zn uptake by plants. We expected that *Glomus mosseae* will help for Zn uptake to horticultural vegetable plant. Our findings showed that onion, garlic, chickpea, broad bean, carrot plants Zn uptake significantly ($p \leq 0.05$) increased due to mycorrhizae amendment in the experimental field (Table 8). Similarly, other study found that maize plants inoculated with AMF took up more Zn than non-mycorrhizal maize plants (Jansa et al. 2003). They also have found that AMF transported significant amount of leveled Zn even within 15 cm distance from the root hyphae. They speculated that the uptake and translocation of Zn to the maize plants occurred by the external hyphae of AMF. This highlights the direct role of AMF in Zn uptake and transfer to plants. The interactive effect of AMF and P on Zn uptake differed plant to plant. The radish plant tissue Zn concentration reduced 36 to 33 ppm when P was added with AMF. Similarly, parsley plant tissue Zn concentration reduced 27 to 22 ppm when P was also added with AMF (Table 8). A study found that AMF with P fertilizations significantly reduced Zn concentrations of soybean (*Glycine max* Mer.) plant.^[47] They speculated that reduction in Zn concentration in soybean were the result of a dilution effect and could be attributed to increased plant size rather than increased P fertility in soil. A study also concluded that high P inhibition of mycorrhizal activity was the main reason for decreased Zn uptake by crop plant (Lambert et al. 1979). In contrast, the interactive effect of AMF and P significantly ($p \leq 0.05$) increased Zn uptake of broad bean and cress plant tissue (Table 8). Similar findings observed from other study as well (Kizhaeral et al. 2014). They found that AMF inoculated maize plants

had significantly higher Zn concentration irrespective to 60 mg P kg ha⁻¹ application. These results suggested that mycorrhizae can enhance Zn supply to the inoculated plant through improving uptake especially P.

Conclusion

Under field conditions the horticultural vegetable plants used in this study showed better growth and yield response with mycorrhizal inoculation. In generally for all plant species P fertilizer amendment increased plant yield. Also, chickpea had high inoculation effectiveness under field conditions, plants depend not only on mycorrhizal inoculation but also on P supply. Inoculation effectiveness was found to decrease with P fertilization. The results are also demonstrated that mycorrhizae can be used as supplement of P fertilization. Mycorrhizae amendment in the field can successfully infect plant roots, resulting in better plant growth and nutrient (P and Zn) uptake. Thus, AMF symbiosis facilitates the availability of both P and Zn in the soil solution. The synergistic interaction between the two nutrients may assist in enhance uptake of Zn and P which eventually get remobilized into developing plant productivity.

It can be concluded that under less P-level conditions horticultural plant species responding to mycorrhizal inoculation. Further researches should be focused on the study of both physiological and nutritional (other than P and Zn nutrition) factors, which may have caused the improvement of yield of plants grown in AMF inoculated soil, under field condition.

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