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EFFECT OF MYCORRHIZA AND THE PHOSPHORUS CONTENT IN A NUTRIENT SOLUTION ON THE YIELD AND NUTRITIONAL STATUS OF LETTUCE GROWN ON VARIOUS SUBSTRATES*

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Abstract

The potential of mycorrhization of lettuce in hydroponic conditions was tested in a two-year experiment carried out in the autumn of 2011 and 2012 in an unheated foil tunnel. The experimental factors were as follows: inoculation of plants with arbuscular mycorrhizal fungi (AMF+ or AMF-), concentration of phosphorus in a nutrient solution (10 or 40 mg dm^{-3}) and the type of substrate (rockwool or coconut coir). The inoculation of plants with arbuscular mycorrhizal fungi of the genus *Glomus* was performed twice, i.e. while sowing seeds and when planting lettuce plants into mats. The impact of the experimental factors on the degree of colonization of lettuce roots by AMF as well as the yield and nutritional status of plants, expressed as the content of macro- and micronutrients, was studied. The mycorrhizal frequency in roots inoculated with AMF reached 51.36%. The nutrient solution concentration of phosphorus and the type of substrate did not have any significant effect on the mycorrhizal frequency. There were no differences in the yield and content of macronutrients in lettuce leaves between plants inoculated and not inoculated with AMF (AMF+, AMF-). Likewise, there were no differences in the yield caused by different phosphorus levels in the nutrient solution or the types of substrate. Plants growing on rockwool accumulated significantly less P, Ca and Mg in comparison to plants growing on coconut coir. Regarding Ca, this effect was especially pronounced in plants receiving the nutrient solution with a higher concentration of P, i.e. 40 mg dm^{-3}. Mycorrhiza had a significant effect on the content of Cu, Mn and Zn in lettuce leaves. Inoculated plants were characterized by a lower content of these micronutries compared to plants grown without AMF. With respect to Mn, this effect was particularly evident in plants grown on rockwool.

Keywords: *Lactuca sativa,* arbuscular mycorrhizal fungi, soilless culture.

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INTRODUCTION

Arbuscular mycorrhiza is the most common type of mycorrhizae in plants. Arbuscular mycorrhizal fungi (AMF) live in symbiosis with at least 90% of plant species, including most horticultural crops. The characteristic structures of this type of mycorrhiza are arbuscules, i.e. bushy branched ends of AMF hyphae, which are located in cells of plant roots. Arbuscules are involved in the metabolic exchange between a plant and the fungus (Głuszek et al. 2008). Owing to such symbiosis with AMF, the plants are better fed, watered and more tolerant to biotic and abiotic stress factors, such as heavy metals, soil salinity, drought, pathogenic fungi and nematodes. AMF also stimulate the hormones regulating the plant growth and accelerate the rate of photosynthesis (Al-Karaki 2006).

The beneficial effect of AMF on plant growth has been proven in the traditional cultivation of lettuce, tomato, onion and hot pepper (Sylwia et al. 2001, Schroeder, Janos 2004). On the other hand, using AMF in soilless cultures is still open to investigations. The results of previous studies (Al-Karaki 2006, Dasgan et al. 2008, Cwala et al. 2010, Baslam et al. 2011*a*,*b*) have indicated the potential of AMF to colonize roots of plants in soilless cultures. However, benefits for plants resulting from the symbiosis have not been clearly identified. Baslam et al. (2011*a*) demonstrated that AMF improved the growth and nutritional quality of lettuce grown in a greenhouse. A positive effect of AMF on yield was also observed in the cultivation of tomato (Dasgan et al. 2008) and pepper (Ikiz et al. 2009). On the other hand, Mueller et al. (2009) and Maboko et al. (2013) showed that arbuscular mycorrhiza did not have any effect on the nutrient uptake, growth and yield of tomato grown in soilless culture. The reason for these discrepancies is probably the degree of colonization of plant roots by AMF in hydroponic cultures, which depends i.a. on the P concentration in the root zone of plants and the type of substrate (Ryan, Graham 2002, Ikiz et al. 2009, Cwala et al. 2010, Maboko et al. 2013).

The objective of the study was to determine the effect of AMF inoculation, different phosphorus concentrations in a nutrient solution and the type of substrate, i.e. rockwool and coconut coir, on the colonization of plant roots by AMF, and on the yield and nutritional status of lettuce.

MATERIAL AND METHODS

In the autumn of 2011 and 2012, a two-year experiment was conducted at the University of Agriculture in Krakow. Lettuce plants (*Lactuca sativa* L.) cv. Melodion were grown in a foil tunnel, in rows filled with coconut coir or rockwool mats. The following were taken as experimental factors: the concentration of P in a nutrient solution (10 or 40 mg dm^{-3}), inoculation with AMF or no inoculation (AMF+ or AMF-) and the type of substrate (rockwool Grotop Master Dry, Grodan® or coconut coir mate Forteco Profit, Van der Knapp®). The experiment consisted of 8 treatments, each in triplicate. There were 15 plants in each replication.

Lettuce seedlings were grown in small rockwool cubes. At the time of sowing seeds into the cubes, some batches of seeds were inoculated with an inoculum containing *Glomus mosseae* and *Glomus intraradices* (v/v 1:1; 720 propagules per 1 g). The inoculation was performed by introducing the inoculum into holes for seeds in the rockwool cubes. Lettuce seedlings at the stage of four leaves were transplanted into cultivation rows filled with substrates remaining after the spring and summer cultivation of tomato. During the cultivation of tomato, half of the substrates (coconut coir and rockwool) were incoulated with an inoculum containing fungi species of the genus *Glomus* (*G. mosseae, G. intraradices, G. aggregatum, G. etunicatum, G. deserticola, G. clarum, G. monosporum).* The effectiveness of mycorrhization of tomato roots grown in substrates used in that experiment was verified. Lettuce grown in cultivation rows received a nutrient solution with the same content of macro- and micronutrients (mg dm^{-3}) except P, i.e. N – 150, K – 200, Mg – 40, Ca – 170, Fe – 1.50, Mn – 0.60, Zn – 0.50, B – 0.33, Cu – 0.10. The nutrient solution was applied by drip irrigation in an open cycle (without recirculation). The frequency of fertigation was adjusted to the phase of plant growth and substrate moisture.

Lettuce was harvested just once, on day 46 from the setting of plants into the mats. The chemical analysis of the plant material was carried out on five representative heads of lettuce, collected separately from each replication of every experimental treatment. A quarter of each lettuce head was chopped in a blender. The content of dry matter was determined in the material thus obtained. The remaining leaves were dried at 65°C (24 h) in a laboratory dryer with forced air circulation in order to determine the content of macro- and micronutrients. Dried samples were ground in a variable speed rotor mill Pulverisette 14 (FRITSCH) using a 0.5 mm sieve. The content of P, K, Ca, Mg, S, B, Cu, Fe, Mn, Mo and Zn in leaf samples was determined after mineralization in 65% extra pure HNO_3 in a microwave system CEM MARS-5 Xpress (Pasławski, Migaszewski 2006), using a high-dispersion spectrometer ICP-OES (Inductively Coupled Plasma Optical Emission Spectrometry) Prodigy Teledyne Leeman Labs. The N content in leaves was assayed by the Kjeldahl method in a VELP Scientifica UDK 193 distillation unit.

Isolation of the root system of plants took place during the harvest. Three plants from each treatment were selected for the isolation. 10 g samples were taken from the isolated root system and then microscopic slides were prepared from this material according to a modified Philips, Hayman (1970) method. Collected roots were cold macerated in a solution of 10% KOH (12 h), rinsed in water and acidified with 5% lactic acid (12 h). Because of the delicate structure of lettuce roots, the maceration and acidification time was limited to 12 hours. Then, the roots were stained in 0.03% trypan blue solution dissolved in a mixture of lactic acid, glycerol and water (v/v/v 1:1:1). After staining, the roots were cut into 1 cm pieces, from which microscopic slides were prepared. They were encapsulated in a mixture of glycerol and lactic acid (v/v 5:1). Each treatment was represented by 90 pieces of roots, each having a length of 1 cm. The colonization of roots by arbuscular mycorrhizal fungi was assessed under a microscope Axio Imager N2 (Carl Zeiss) with the use of the Nomarski contrast, according to the method developed by Trouvelot et al (1986). Mycorrhizal frequency (F%), i.e. the frequency of appearance hyphae in a root, was estimated in each root segment.

The results were subjected to a three-way analysis of variance. Statistical calculations of the results were performed with the Anova module of Statistica 10.0 PL at $P < 0.05$. Whenever some changes occurred, significant homogenous groups were distinguished by the Tukey test. Similar results were obtained in the both years of the research. Consequently, the results are presented as the means of the data acquired in 2011 and 2012.

RESULTS AND DISCUSSION

Mycorrhizal analysis

Regardless of the concentration of P in the nutrient solution or the type of substrate, the average mycorrhizal frequency (F%) in plants inoculated with AMF was 51.36%. No presence of mycorrhiza was observed (F%=0) in the roots of plants grown in substrates without the inoculum (Table 1). There were no significant effects of the other experimental factors, i.e. the nutrient solution P concentration or the type of substrate on mycorrhizal

Table 1

Effect of P level in nutrient solution (mg dm^{-3}), mycorrhiza and substrate on mycorrhizal frequency in roots of lettuce

Treatement	Mycorrhizal frequency (%)
Phosphorus level	
10	25.29±7.79
40	26.09±7.95
	n.s.
Mycorrhiza	
AMF-	0.00±0.00
AMF+	51.36±2.03
	*
Substrate	
coconut	24.66±7.56
rockwool	26.70±8.17
	n.s.

* means are significantly different; n.s. – differences are not significant; ± – standard error; AMF-/AMF+ – non-inoculated plants by arbuscular mycorrhizal fungi (AMF)/inoculated plants by AMF

frequency. The degree of the AMF colonization of lettuce roots detected in our experiment is lower than described by Baslam et al. (2011*a*), who cultivated lettuce in a mixture of vermiculite, sand and peat, obtaining the root colonization in a range of 56 to 61% when a commercial inoculum containing *G. mosseae* and *G. intraradices* had ben applied to the growing medium, and 65% in roots of plants inoculated with *G. fasciculatum*. The reason for the varying degree of the colonization of plant roots by AMF may be the concentration of P in a nutrient solution and the type of substrate (Cwala et al. 2010, Maboko et al. 2013). The current experiment did not prove any significant effect of the P concentration in the nutrient solution on the mycorrhizal frequency. The plants receiving the nutrient solution with the standard concentration of P (40 mg dm^{-3}) were characterized by the mycorrhizal frequency (F%) of 25.27%, while the ones receiving the nutrient solution with the lower tested P concentration (10 mg dm^{-3}) were characterised by F% equal 26.09% (Table 1). These results contradict the ones obtained by Schmidt et al. (2010), who grew marigold inoculated by fungi species of the genus *Glomus*. They observed a lower mycorrhizal frequency in roots of plants receiving a nutrient solution with a higher concentration of P. A high P concentration leading to a lower degree of the colonization of plant roots by AMF was also demonstrated by Ikiz et al. (2009) in the cultivation of pepper and by Cwala et al. (2010) on tomatoes. According to Amijee et al. (1989), a high concentration of absorbable P in soil reduced the degree of root colonization, which is an effect of the marked change in plant or fungal physiology.

Substrates used in soilless cultivation of plants are characterized by various physicochemical properties (air capacity, content of chemical compounds, etc.), which may have an impact on the colonization of plant roots by AMF. Maboko et al. (2013) suggest that the content of phenolic compounds, lignin and other organic compounds in coconut does not create favourable conditions for the development of AMF, which may have some influence on the degree of colonization of roots by AMF. In our experiment, the coconut substrate was not observed to have had a negative impact on the development of mycorrhiza, which was confirmed by similar mycorrhizal frequency values calculated for roots of lettuce grown in rockwool and coconut coir (26.70% and 24.66%, respectively) – Table 1. The current experiment seems to indicate that both rockwool and coconut coir may be conducive environments for the development of mycorrhiza.

Yield

The yield of lettuce, expressed as the weight of a head, did not depend on the experimental factors, i.e. the concentration of P in the nutrient solution, type of substrate and inoculation with AMF (Table 2). The lack of an effect of AMF on the yield of lettuce could have been due to the low intensity of the formation of arbuscules. The low intensity of the creation of arbuscules means that there is no exchange of substances between the plant and the

Table 2

Effect of P level in nutrient solution (mg dm^{-3}), mycorrhiza and substrate on yield of lettuce expressed as a weight of head

Treatement	Yield (g)
Phosphorus level	
10	372.56±5.34
40	361.77±7.51
	n.s.
Mycorrhiza	
AMF-	366.64±5.26
AMF+	367.69±7.91
	n.s.
Substrate	
coconut	366.65±5.73
rockwool	367.67±7.52
	n.s.

* Explanations see Table 1

fungus, which can limit the benefits generated by mycorrhizae, including better yields. In this experiment, the relative abundance of arbuscules (%), determining the relative abundance of arbuscule formation within an entire sample, was very low level (0.25% in plants subjected to the inoculation with AMF), regardless of the nutrient solution P concentration and the type of substrate. The limited effect of AMF on the yield of plants was also reported by Maboko et al. (2013) in the cultivation of tomato on sawdust and coir. No effect of AMF on the yield of plants despite a high mycorrhizal frequency (77.7-78.2%) was attributed to the presence phenolics, lignin and other organic compounds in the used substrates, which could reduce the development of mycorrhizal structures. Similarly, Mueller et al. (2009) did not observe the effect of mycorrhiza on the yield of tomato grown in sand and peat. These results contrast with the ones of Dasgan et al. (2008), who obtained a higher yield of tomato from plants subjected to the inoculation with *G. fasciculatum* than from plants grown without AMF. The authors suggested that it was the consequence of the inoculated tomato plants being able to use effectively photoassimilates for fruit production instead of vegetative growth.

Mineral status of plants

Inoculation with AMF did not have any significant effect on the content of macroelements in lettuce leaves (Table 3). However, mycorrhiza significantly affected the content of some microelements, e.g. Cu, Mn and Zn. The inoculated plants were characterized by a lower concentration of these elements than plants grown without AMF (Table 4). By increasing the absorption surface of plant root systems, AMF improve the uptake of water and nutrients, which leads to an improved nutritional status of plants. Generally, we did not prove any significant effect of AMF on the nutritional status of plants expressed as the content of mineral compounds in leaves, with the exception of Cu, Zn and Mn. Likewise, Cwala et al. (2010) did not observe any improvement of the nutritional status of plants colonized by AMF com-

Table 3

Effect of P level in the nutrient solution (mg dm^{-3}), mycorrhiza and substrate on dry matter and content of macroelements in lettuce

Treatement	Dry matter (%)	N	P	K	Ca	Mg
		(% d.m)				
Phosphorus level 10 40	4.26±0.17 4.24±0.17 n.s.	4.83±0.05 4.81±0.03 n.s.	0.79±0.02 0.98±0.02 *	8.96±0.10 8.48±0.16 *	1.74±0.04 1.67±0.07 n.s.	0.49±0.00 0.50±0.01 n.s.
Mycorrhiza AMF- AMF+	4.16±0.13 4.33±0.16 *	4.87±0.04 4.77±0.03 n.s.	0.89±0.03 0.87±0.03 n.s.	8.87±0.14 8.59±0.15 n.s.	1.72±0.06 1.69±0.06 n.s.	0.49±0.01 0.50±0.01 n.s.
Substrate coconut rockwool	4.13±0,09 4.36±0,14 *	4.77±0.04 4.87±0.04 n.s.	0.92±0.03 0.84±0.03 *	8.86±0.13 8.60±0.16 n.s.	1.87±0.02 1.54±0.04 *	0.52±0.01 0.46±0.01 *

* Explanations see Table 1

Table 4

Effect of P level in the nutrient solution (mg dm^{-3}), mycorrhiza and substrate on content of microelements in lettuce

Treatement	Fe	Cu	Zn	Mn	Mo	B
	(mg kg^{-1} d.m.)					
Phosphorus level 10 40	137.87±2.89 120.73±1.95 *	4.88±0.27 4.68±0.30 n.s.	49.94±1.78 41.39±1.53 *	67.39±3.91 61.26±2.50 *	0.58±0.02 0.57±0.04 n.s.	31.70±0.30 30.27±0.51 *
Mycorrhiza AMF- AMF+	132.22±3.50 126.38±3.42 n.s.	5.13±0.29 4.43±0.23 *	48.78±1.90 42.55±1.86 *	71.08±2.63 57.57±2.83 *	0.59±0.01 0.56±0.05 n.s.	30.77±0.42 31.20±0.52 n.s.
Substrate coconut rockwool	131.12±2.71 127.48±4.19 n.s.	3.96±0.12 5.60±0.16 *	45.19±2.19 46.14±2.00 n.s.	65.56±1.41 63.08±4.58 n.s.	0.55±0.02 0.60±0.04 n.s.	31.91±0.26 30.06±0.48 *

* Explanations see Table 1

pared to non-inoculated plants. Those authors suggested that the lack of diversity in the content of macro- and micronutrients in plants, despite the presence of AMF, could have been caused by the nutrient rich environment of the plant root zone in hydroponic conditions. Dasgan et al. (2008) and Maboko et al. (2013) did not demonstrate an increased nutrient uptake by plants inoculated with AMF compared to plants grown without the presence of mycorrhiza. In our study, the limited effect of AMF on the nutrient uptake by plants may have stemmed from the low intensity of the formation of arbuscules (0.25%). The low level of the intensity at which arbuscules are formed means that there is no exchange of substances between the plant and the fungus, which is why the nutritional status of plants does not improve.

In our experiment we observed a significant reduction in the concentration of Zn, Cu and Mn in the leaves of inoculated plants compared to plants grown without AMF. However, these values were located in the range which is considered as the optimal concentration of micronutrients in lettuce leaves (Barker, Pilbeam 2007). Chen et al. (2003) and Christie et al. (2004) demonstrated experimentally that, as well as protecting plants against excessive Zn uptake in Zn-contaminated soils, AMF could improve the zinc uptake by plants growing in zinc-deficient soil. Joner et al. (2000) indicated that the surface area of AMF serves as adsorptive sites for heavy metal cations and this process prevents entry of toxic metals into the host plants. In hydroponic cultures, despite supplying plants with a standard nutrient solution, an increase in the concentrations of minerals in the root zone of plants has been frequently observed as an effect of accumulation (Jarosz et al. 2011, Kleiber 2012). It can be assumed that the limiting effect of AMF on the uptake of Zn and Cu by mycorrhized plants was the result of elevated concentrations of these components in the root zone.

The concentration of P in the nutrient solution, regardless of the type of substrate and AMF inoculation, had a significant impact on the content of P and K in lettuce leaves. Regardless of the type of substrate and inoculation with AMF, a higher P content was assessed in leaves of plants receiving the nutrient solution with 40 mg P dm^{-3} than in plants treated with the nutrient solution containing 10 mg P dm^{-3}. In contrast, a reverse relationship was demonstrated for the K content in lettuce leaves. The differentiation of the P concentration in the nutrient solution also affected the content of some microelements such as Fe, Zn, Mn and B in lettuce leaves. Significantly less of these components was determined in leaves of plants receiving the nutrient solution with more P, i.e. 40 mg dm^{-3}, than in leaves of plants supplied the nutrient solution with 10 mg P dm^{-3}. The higher content of Zn in leaves of lettuce fed with the nutrient solution containing less P may be explained by the P and Zn antagonism that appears when those elements exceed their threshold values (Shetty et al. 1994). Our results are in agreement with Zhu et al. (2001), who observed that an increase in P availability caused a significant reduction of the Zn uptake and concentration of Zn in tissue of wheat cultivated in sand. Analogously, Haldar, Mandal (1981) proved that an application of P caused a decrease in concentrations of Zn, Cu, Fe and Mn in shoots of rice.

The content of P, Ca and Mg in lettuce leaves was significantly dependent on the type of substrate. Irrespective of the level of P in the nutrient solution and the AMF inoculation, plants grown on rockwool accumulated less of these elements than plants grown on coconut coir (Table 3). The interaction of the experimental factors, i.e. the concentration of P in the nutrient solution and type of substrate, had an impact on the content of Ca in lettuce leaves (Figure 1). Plants grown on coconut coir had the highest concentration of Ca, and this relationship was demonstrated at both levels of P in the nutrient solution. In the cultivation on rockwool, an application of the nutrient solution with 40 mg P dm^{-3} was the least preferred combination (the lowest concentration of Ca). The type of substrate also affected the content of Cu and B in lettuce leaves (Table 4). Regardless of the level of P in the

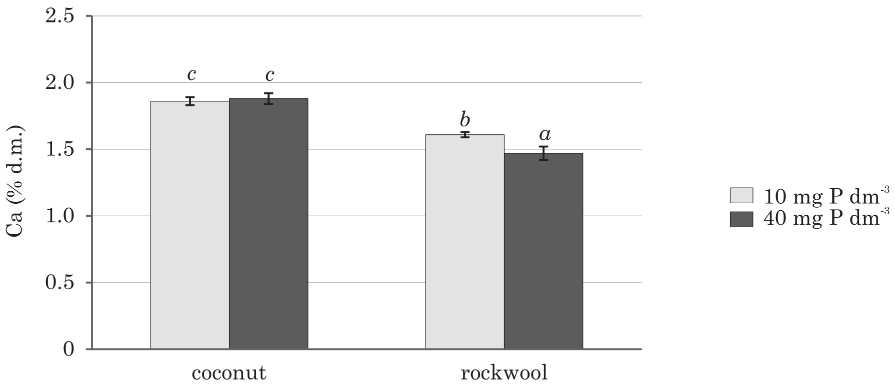

Fig. 1. Interaction between P level and substrate on the content of Ca in leaves of lettuce. Means followed by different letters differ at $P < 0.05$; bars indicate standard error

nutrient solution and the AMF inoculation, a significantly higher content of Cu was found in leaves of plants grown on rockwool compared to plants grown on coconut coir. A contrary relationship was proven for the B content in leaves. Other findings were as folows: a significant effect of interaction of the experimental factors such as the P level in a nutrient solution and the type of substrate on the content of B (Figure 2), interaction of AMF inoculation and the type of substrate (Figure 3) and an interaction of all the three experimental factors with the content of Mn in lettuce leaves (Figure 4).

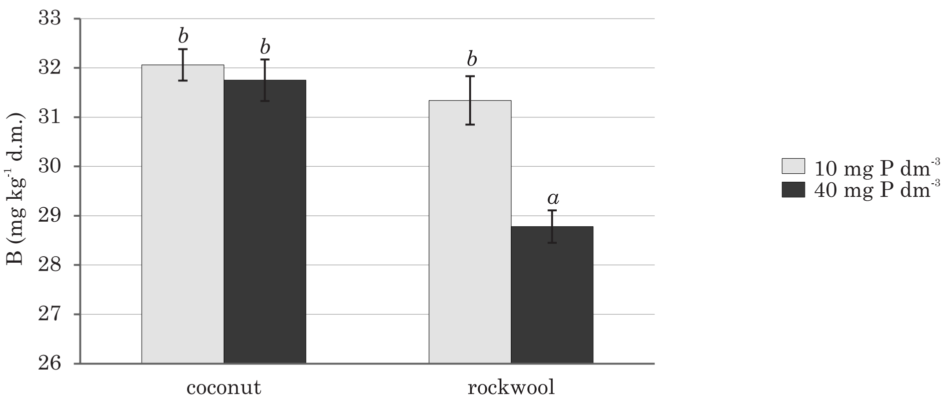

Fig. 2. Interaction between P level and substrate on the content of B in leaves of lettuce. Means followed by different letters differ at $P < 0.05$; bars indicate standard error

CONCLUSIONS

1. Colonization of mycorrhiza of lettuce plants grown in soilless culture was demonstrated; the level of P in a nutrient solution and the type of substrate did not influence the frequency of mycorrhiza.

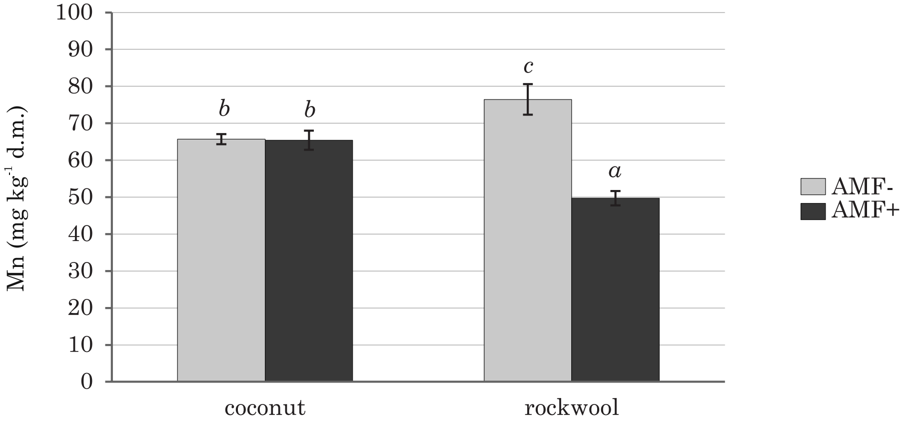

Fig. 3. Interaction between AMF inoculation and substrate on content of Mn in leaves of lettuce. Means followed by different letters differ at $P < 0.05$; bars indicate standard error

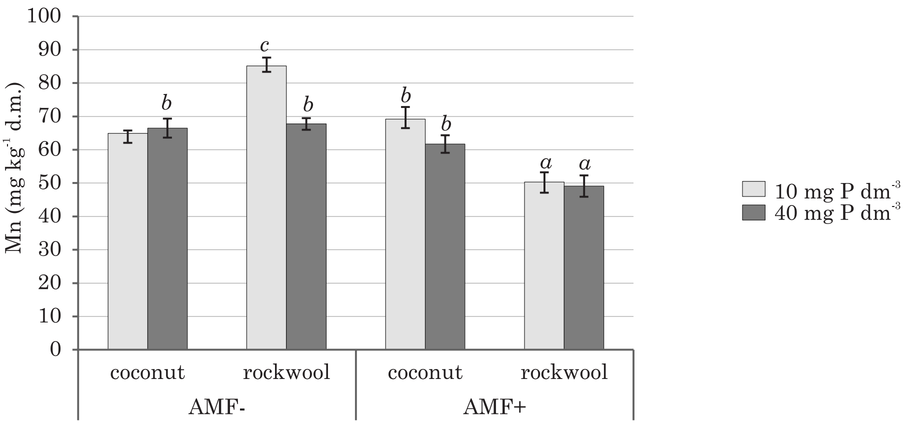

Fig. 4. Interaction between P level, mycorrhiza and substrate on the content of Mn in leaves of lettuce.
Means followed by different letters differ at $P < 0.05$; bars indicate standard error; AMF-/AMF+ – non-inoculated plants by arbuscular mycorrhizal fungi (AMF)/inoculated plants by AMF

2. Neither the P level in a nutrient solution, the type of substrate nor the AMF inoculation affected the lettuce yield.

3. Mycorrhiza with AMF did not affect the nutrient status of plants, except for Cu, Zn and Mn. Less Cu, Zn and Mn was found in mycorrhized plants.

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