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Effect of mycorrhizae of pine seedlings on the utilization of different mineral phosphorus sources

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Pine seedlings (*P. sylvestris* L.) growing on the unsterilized sand, with addition of different phosphorus sources, were inoculated with ectomycorrhizal fungi. Lack of P in the substrate restricted mycorrhizal infection of roots. In the presence of $AlPO_4$ and $FePO_4$ inoculation with *A. verna* and *H. mesophaeum* have positive effect on the seedlings growth and survival. Strain of *H. mesophaeum* intensified the phosphorus uptake, particulary when $FePO_4$ was applied.

INTRODUCTION

Participation of mycorrhizae in the mineral phosphorus cycle in forest trees has been the subject of numerous studies for a long time. There, it has been proved that mycorrhizae increase host plant ability of phosphorus nutrition (McComb 1938; Harley 1959; Lundeberg 1961; Ritter, Lyr 1965; Mejstrik, Krause 1973; Mejstrik 1975). The analyses with ^{32}P indicate unequivocally that ectomycorrhizal mycelia are capable of phosphorus uptake from the substrate and of its transfer through ectomycorrhizae to the pine roots (Melin, Nillson 1950). Studies of Bowen and Theodorou (1967) and those of Mejstrik and Krause (1975) have shown that differences in the phosphorus uptake depend upon fungal symbiont. Gawliński (1979, 1983) has observed that pine seedlings have been capable of phosphorus uptake from poorly soluble compounds; however, he has not taken into account the influence of mycorrhizal fungi.

Soil mycoflora is considered important in the phosphorus release from poorly soluble compounds, although effect of mycorrhizae on the uptake of this element has not been studied in detail. Voigt (1971) discusses this problem in the context of V-A mycorrhizae. Pachlewski and Chruściak (1981) stated that ectomycorrhi-

zal mycelia are capable of utilization as a phosphorus source poorly soluble phosphates.

In the studies on the effect of pine mycorrhizae on the phosphorus uptake from different mineral compounds the authors have given the particular attention to the capability of some symbiotic fungi to utilize poorly soluble aluminium and iron phosphates. These compounds are abundant in acid soils, such as the forest soils.

MATERIAL AND METHODS

Pot experiments: pine seedlings and fungi were grown in pots filled with unsterilized quartz sand (not more than 17 kg per pot). The sand was enriched with nutrient solutions. They contained: NH_4NO_3 — 0,68 g, KCl — 0,28 g, $CaCl_2 \cdot 6H_2O$ — 1,3 g, $MgSO_4 \cdot 7H_2O$ — 0,97 g; or in exchange KH_2PO_4 — 0,26 g, 18% P_2O_5 superphosphate — 0,76 g, 29% P_2O_5 ground phosphate rock — 0,45 g, $AlPO_4$ — 0,3 g, $FePO_4$ — 0,36 g in 1 liter of water. The main MPKCaMg nutrient composition was supplemented with solution of microelements: H_3BO_4 — 5 g, $MnSO_4$ — 5 g, $CzSO_4$ — 0,1 g, $ZnSO_4$ — 0,1 g, $MoNH_4$ — 0,25 g, and 0,5 ml of 1% iron citrate and

Table 1

Experiment plan

P source	Fungal inoculum			Control
	Amanita verna	*Hebeloma mesophaeum*	MrgX I strain	
	Pots number			
Without P	19,20,21	37,38,39	1,2,3	55,56,57
KH_2PO_4	22,23,24	40,41,42	4,5,6	58,59,60
Superphosphate —18% P_2O_5	25,26,27	43,44,45	7,8,9	61,62,63
Ground phosphate rock —29% P_2O_5	28,29,30	46,47,48	10,11,12	64,65,66
$AlPO_4$	31,32,33	49,50,51	13,14,15	67,68,69
$FePO_4$	34,35,36	52,53,54	16,17,18	70,71,72

refilled with distilled water to 1000 ml. Dose of 5 ml per pot was used. Additional enrichment with nitrogen was done in May 1982 and 1983 (300 mg of N per pot); 50% of water capacity of the sand was maintained by pouring of distilled water. Post-culture pH equaled 4,4-4,8. The investigation were held in fully-glassed wegetative chamber.

In the studies scots pine seeds were used. Seeds were sown (50 per pot) 2 weeks after nutrient enrichement of the substrate. After plant germination on the 11th

of May 1981 number of seedlings was reduced to 30 specimens per pot. Next year only 12 plants per pot were left to grow.

Two species of ectomycrorhizal fungi, *Amanita verna* (Bull.: Fr.) Pers. ex Vitt. (no 0141) and *Hebeloma mesophaeum* (Pers.: Fr.) Quél. (no 3037), and ectendomycorrhizal pine strain (MrgX I) were tested. Fungal inocula were isolated from 4-6 weeks, cultures on PP liquid medium (Pachlewski, Pachlewska 1974). Fungal suspension in water was introduced into small grooves between seedling rows, at the depth of 2 cm. Then grooves were filled with sand and moistened with distilled water. Inoculation was performed on the 12th, 15th and 23rd of June 1981. Phosphorus combinations without inoculation were the control for the test; 3 replications of each experiment treatment were done. In total 72 pots were analysed during 3 growing seasons. In December 1983 (the end of the experiment) shoots were measured, roots taken for mycorrhizal analysis, and needles and roots for chemical analysis of NPKCaMg content. Dry weights of shoots, roots and needles were also determined.

Cultures in vitro: from the earlier studies (Pachlewski, Chruściak 1981) it may be inferred that, in vitro, cultures $AlPO_4$ and $FePO_4$ were for some mycorrhizal fungi very good phosphorus source. Due to that *H. mesophaeum* (no 3037) strain was also tested similarly. It was grown on PP medium where KH_2PO_4 was replaced by $AlPO_4$ (64 mg per 1) and $FePO_4$ (80 mg per 1). Culture on PP medium with a full composition was the control. All the cultures were conducted on Petri dishes in 3 replications and incubated at 25°C for 21 days.

RESULTS

Results of the studies on pine mycorrhizae under different "phosphorus conditions" showed that investigated phenomenon is variable in the case of investigated fungi, as well as fungi which were aboriginal for the culture substrate. Significant difference in mycorrhizal infection of seedling roots which occurred in the control treatment (no inoculation) dependend on the phosphorus source. It was the clearest in the combinations without P and with $FePO_4$ where mycorrhizae of aboriginal fungi were absent, or nearly absent. In the combinations with superphosphate relatively welldeveloped mycorrhizae occurred, although their number varied with pot. In other phosphorus combinations without inoculation all pots were similar — pine seedlings were characterized by ectendomycorrhizae with poor intracellular infection (Table 2).

The results of the analysis of different P source influence on forming of the mycorrhizae clearly indicated the negative effect of the lack of phosphorus in the culture medium. It was evident in a number of the inoculation treatments. In all combinations without P, except inoculation with *H. mesophaeum*, all analysed fungi infected seedlings roots and formed mycorrhizae only sporadically (Table 2). Negative effect of phosphorus lack was marked both in mycorrhizae structure and their

Table 2

Influence of different phosphorus sources on pine mycorrhizae

P source	Inoculum	Ectomycorrhizae			Ectendomycorrhizae MrgX type %	Total %
		Amanita verna %	*Hebeloma mesophaeum* %	others %		
Without P	*A. verna*	8	—	0	3	11
	H. mesophaeum	—	25	0	0	25
	MrgX	—	—	0	3	3
	control	—	—	0	5	5
KH_2PO_4	*A. verna*	35	—	10	20	65
	H. mesophaeum	—	48	0	21	69
	MrgX	—	—	5	5	10
	control	—	—	0	45	45
Superphosphate —18% P_2O_5	*A. verna*	18	—	11	6	35
	H. mesophaeum	—	10	3	38	51
	MrgX	—	—	35	18	53
	control	—	—	49	0	49
Ground phosphate rock —29% P_2O_5	*A. verna*	25	—	30	2	57
	H. mesophaeum	—	10	26	35	71
	MrgX	—	—	38	5	43
	control	—	—	25	10	35
$AlPO_4$	*A. verna*	11	—	25	5	41
	H. mesophaeum	—	25	22	3	50
	MrgX	—	—	38	25	63
	control	—	—	3	30	33
$FePO_4$	*A. verna*	18	—	3	2	23
	H. mesophaeum	—	62	2	0	64
	MrgX	—	—	10	2	12
	control	—	—	0	0	0

earlier senescence. In other phosphorus combinations mycorrhizae relationships in medium with $FePO_4$ and $AlPO_4$ should be given much attention. Test with *H. mesophaeum* and *A. verna* (Table 2) showed that these mycorrhizal fungi are capable of utilization as a phosphorus source both mentioned above compounds. This corresponded with test in vitro for the growth of vegetative mycelium of *H. mesophaeum* (Fig. 1). The positive effect of *H. mesophaeum* and *A. verna* ectomycorrhizae resulted not only in better seedling growth, due to fungal transfer of P to host plant (Table 4,5), but also in seedling survival under difficult conditions of phosphorus nutrition (Fig. 2, 3, 4). The results of experiment on ectendomycorrhizal strain MrgX I did not elucidate its mycorrhizal reaction under investigated phosphorus conditions. This was probably related to inoculation success.

Mycorrhizal infection of aboriginal fungi, especialy *Rhizoctonia silvestris* sensu

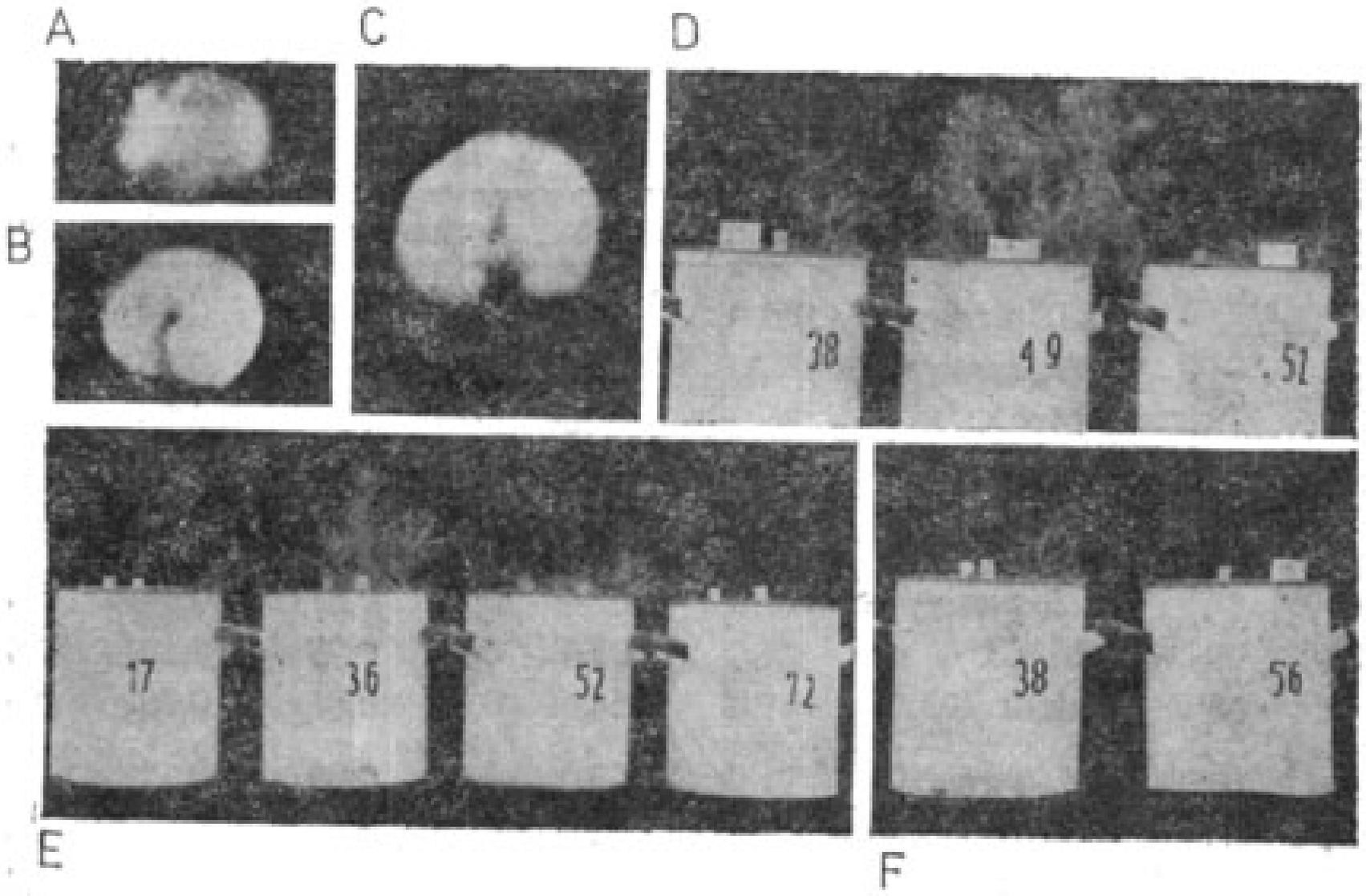

Fig. 1. Mycelium growth of *Hebeloma mesophaeum* in vitro (*A*, *B*, *C*) and Pine seedlings growth (*D*, *E*, *F*)

A — with KH_2PO_4; *B* — $FePO_4$; *C* — $AlPO_4$ (on Pp medium); *D* — without P (pot 38); with $AlPO_4$ (p. 49); with $FePO_4$ (p. 52) inoculated with *H. mesophaeum*; *E* — on the substrate with $FePO_4$, inoculated; Mrg X I strain (p. 17); *A. verna* (p. 36); *H. mesophaeum* (p. 52); without inoculation (p. 72); *F* — on the substrate without P: inoculated with *H. mesophaeum* (p. 38) and without inoculation (p. 56)

Levisohn (1963), obscured results of the studies on the role of analysed fungi in the utilization of different sources of phosphorus. It was caused by the use of the unsterile culture medium. Unsterilized sand as a substrate made adaptation of inoculated tested species, especially MrgX I strain, more difficult. Thus, the forming of mycorrhizae varied between replications of a particular combination. Therefore, the results of the studies on mycorrhizae should be confirmed in further experiments.

In case of phosphorus nutrition of pine it may be inferred that seedlings growth was good in inoculated combinations with well-soluble phosphorus compounds such as KH_2PO_4 and superphosphate (Table 3), as well as on substrate enriched with phosphate rock and $AlPO_4$. The latter with the exception of plants inoculated with MrgX I. Yield in pots fertilized with $FePO_4$ was significantly lower than in other treatments. However, plant inoculation with *A. verna* and *H. mesophaeum* made P from $FePO_4$ probably more available for pine.

Changes in the chemical compositions of needles, stems and roots due to the different fertilization and inoculation treatment are given in Table 4. High iron concentration in roots of all combinations inoculated with MrgX I and *A. verna* is worth mentioning. Although nutrient uptake (Table 3) by 3-years, old pine did not show the whole balance (some plants were removed in the course of experiments), it indicated high nutrient utilization under the conditions of the experiment described. The participation of the species of symbiotic fungi, *A. verna* and *H. mesophaeum*, in phosphorus mobilization should be stressed.

Table 3

Influence of P sources and inoculation

P source	Inoculum	% N			% P		
		needles	stems	roots	needles	stems	roots
Without P	*A. verna*	—	—	—	0,04	—	0,03
	H. mesophaeum	3,56	—	—	0,06	—	0,04
	MrgX	—	—	—	0,04	—	0,04
	control	—	—	—	0,03	—	0,04
KH_2PO_4	*A. verna*	1,70	0,59	0,79	0,10	0,03	0,06
	H. mesophaeum	1,57	0,62	0,87	0,08	0,04	0,06
	MrgX	2,03	0,41	0,78	0,10	0,04	0,05
	control	1,54	0,60	0,81	0,08	0,03	0,05
Superphosphate — 18% P_2O_5	*A. verna*	1,59	0,74	0,89	0,12	0,05	0,08
	H. mesophaeum	1,42	0,57	0,96	0,09	0,04	0,06
	MrgX	1,65	0,61	0,96	0,11	0,05	0,07
	control	1,49	0,74	0,95	0,09	0,03	0,07
Ground phosphate — rock — 29% P_2O_5	*A. verna*	2,03	0,89	0,98	0,10	0,05	0,06
	H. mesophaeum	1,53	0,46	0,81	0,07	0,03	0,05
	MrgX	1,54	0,66	0,83	0,10	0,04	0,05
	control	1,51	0,59	0,76	0,07	0,03	0,05
$AlPO_4$	*A. verna*	1,70	0,67	1,02	0,10	0,04	0,07
	H. mesophaeum	1,53	0,60	0,89	0,08	0,04	0,06
	MrgX	1,96	0,81	1,09	0,12	0,05	0,06
	control	1,47	0,52	0,80	0,08	0,03	0,05
$FePO_4$	*A. verna*	3,76	—	2,07	0,08	0,04	0,05
	H. mesophaeum	3,70	—	2,13	0,08	0,08	0,05
	MrgX	—	—	—	0,05	—	0,03
	control	—	—	—	0,04	—	—

CONCLUSIONS

1. Phosphorus lack in the culture medium inhibite mycorrhizal infection of pine seedling roots.

2. Utilization of different sources of mineral phosphorus by pine mycorrhizae depends, to some extent, on the fungal component of mycorrhizae.

3. Pine mycorrhizae with *Hebeloma mesophaeum* and *Amanita verna* enable to use as a phosphorus source poorly soluble phosphates, $AlPO_4$ and $FePO_4$.

4. Under difficult conditions of phosphorus nutrition mycorrhizae positively affect both the balance of this element in host plant, and growth and survival of pine seedlings.

5. In the combinations with phosphorus forms easily available for pine, such

on chemical composition of pine seedlings

% K			% Ca			% Mg			ppm Fe		
needles	stems	roots	needles	stems	roots	needles	stems	roots	needles	stems	roots
0,80	—	0,13	1,61	—	0,20	0,66	—	0,07	215	—	3550
0,82	—	0,34	0,71	—	0,29	0,28	—	0,09	210	—	825
0,84	—	0,14	1,77	—	0,58	0,58	—	0,11	285	—	1050
0,56	—	0,14	1,17	—	0,26	0,40	—	0,09	150	—	735
0,55	0,24	0,27	0,36	0,49	0,20	0,16	0,08	0,07	65	25	1275
0,49	0,25	0,28	0,33	0,52	0,18	0,17	0,09	0,07	55	20	925
0,59	0,25	0,24	0,43	0,45	0,14	0,22	0,10	0,05	70	30	1950
0,52	0,24	0,27	0,45	0,55	0,18	0,18	0,09	0,08	65	35	925
0,41	0,21	0,20	0,51	0,56	0,22	0,17	0,09	0,09	310	55	1025
0,36	0,17	0,18	0,54	0,64	0,21	0,20	0,08	0,06	70	30	850
0,34	0,17	0,17	0,52	0,51	0,14	0,20	0,08	0,07	70	35	2515
0,38	0,20	0,17	0,61	0,65	0,22	0,22	0,10	0,07	75	50	900
0,43	0,26	0,20	0,54	0,64	0,29	0,17	0,10	0,09	75	35	925
0,31	0,16	0,19	0,54	0,61	0,23	0,19	0,09	0,07	70	20	650
0,44	0,18	0,18	0,43	0,61	0,14	0,16	0,09	0,07	60	20	3375
0,31	0,18	0,17	0,49	0,61	0,20	0,15	0,10	0,06	100	40	950
0,45	0,22	0,20	0,42	0,47	0,16	0,18	0,09	0,07	50	60	1175
0,30	0,17	0,20	0,46	0,53	0,13	0,18	0,09	0,07	145	20	925
0,59	0,19	0,28	0,37	0,61	0,13	0,18	0,10	0,07	65	35	715
0,39	0,17	0,17	0,42	0,57	0,15	0,16	0,07	0,06	50	35	625
0,56	0,41	0,46	0,37	0,34	0,26	0,19	0,10	0,10	350	110	875
1,25	0,67	0,58	0,46	0,63	0,25	0,20	0,25	0,12	90	80	800
0,64	—	0,20	1,07	—	0,35	0,35	—	0,11	320	—	1650
0,68	—	—	1,06	—	—	0,43	—	—	300	—	—

Table 4

Nutrients uptake by pine seedlings in the dependence of phosphorus source and inoculation

P source	Fungal inoculum	mg/pot				
		N	P	K	Ca	Mg
1	2	3	4	5	6	7
Without P	*A. verna*	—	0,30	5,19	10,34	4,22
	H. mesophaeum	—	1,02	13,14	11,35	4,38
	MrgX	—	0,24	3,91	8,83	2,78
	control	—	0,23	3,30	6,85	2,23
KH_2PO_4	*A. verna*	534,3	33,65	181,18	159,85	52,10
	H. mesophaeum	527,7	31,36	172,75	152,91	54,14
	MrgX	457,5	26,53	151,81	128,64	50,85
	control	529,6	29,06	181,62	178,02	60,70
Superphosphate — 18% P_2O_5	*A. verna*	479,3	37,37	121,38	174,99	51,56
	H. mesophaeum	588,9	37,60	138,45	235,78	66,42

1	2	3	4	5	6	7
	MrgX	560,5	39,52	114,57	176,77	59,49
	control	538,0	33,36	125.21	223,25	65,81
Ground phosphate rock – 29% P_2O_5	*A. verna*	579,0	31,10	127,55	194,21	52,09
	H. mesophaeum	564,9	29,75	127,98	239,26	67,20
	MrgX	438,5	27,64	115,92	146,15	45,01
	control	615,5	32,64	138,22	246,27	63,10
$AlPO_4$	*A. verna*	584,2	36,33	145,19	154,76	56,35
	H. mesophaeum	571,9	33,83	123,24	176,51	61,42
	MrgX	307,2	18,26	85,76	70,55	26,73
	control	589,1	33,89	150,33	208,85	59,29
$FePO_4$	*A. verna*	171,3	4,47	38,75	24,06	10,04
	H. mesophaeum	110,2	3,22	40,46	19,52	8,54
	MrgX	—	0,39	4,58	7,69	2,50
	control	—	0,14	2,38	3,71	1,50

Table 5

Influence of phosphorus compounds and fungus inoculation on the dry weight and growth of pine

P Source	Fungal inoculum	Dry weight g/pot			Total	height of stem (cm)
		needles	stem	roots		
Without P	*A. verna*	0,62	—	0,18	0,80	4,0
	H. mesophaeum	1,44	—	0,39	1,86	5,2
	MrgX	0,45	—	0,15	0,60	4,2
	control	0,55	—	0,16	0,71	3,7
KH_2PO_4	*A. verna*	18,23	10,98	20,21	49,42	22,5
	H. mesophaeum	16,72	10,95	22,66	50,53	25,5
	MrgX	13,85	9,94	17,40	41,19	33,8
	control	19,75	9,37	20,90	50,02	21,3
Superphosphate – 18% P_2O_5	*A. verna*	18,17	9,35	13,62	41,14	15,2
	H. mesophaeum	21,85	11,96	21,92	55,73	24,4
	MrgX	18,85	10,05	19,64	48,54	27,3
	control	19,75	9,68	18,12	47,55	21,4
Ground phosphate rock – 29% P_2O_5	*A. verna*	16,31	8,70	17,14	42,15	18,6
	H. mesophaeum	21,67	11,68	22,17	55,52	24,5
	MrgX	16,56	8,82	15,10	40,48	32,6
	control	22,86	13,82	24,88	61,66	23,6
$AlPO_4$	*A. verna*	19,27	9,20	19,72	47,59	23,4
	H. mesophaeum	20,21	10,21	22,62	53,05	25,9
	MrgX	9,25	4,15	8,47	21,87	28,5
	control	21,50	14,26	24,85	60,61	22,6
$FePO_4$	*A. verna*	2,60	1,54	3,48	7,62	18,0
	H. mesophaeum	2,07	0,96	1,58	4,61	7,6
	MrgX	0,64	—	0,24	0,88	5,3
	control	0,30	—	0,05	0,35	3,3

as KH_2PO_4 and superphosphate, as well as with ground phosphate rock and $AlPO_4$ — poorly soluble compounds, seedling growth was good both in inoculated and non-inoculated combinations.

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