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ROLE OF VESICULAR-ARBUSCULAR MYCORRHIZA FUNGI AND PHOSPHORUS IN THE CONTROL OF ALFALFA DAMPING-OFF PATHOGENS

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Abstract

The effects of vesicular arbscular Mycorrhiza fungi (*Glomus* sp, *Gigaspora* sp , *Acalospora* sp) and different sources and levels of phosphorus on damping off caused by *Fusarium oxysporum, Verticillium albo-atrum* and *Colletotrichum trifolii* in alfalfa plants were investigated in pot experiments. VAMF decreased damping off in plants grown in soil amended with superphosphate or rock phosphate, whereas damping-off increased in soil unamended with phosphorus source. The extent of mycorrhizal colonization increased by the addition of superphosphate to the soil in comparison with rock phosphate or without phosphate. Dry weight of shoots was affected by using VA mycorrhiza and phosphatic fertilization, where it was increased in the presence of mycorrhiza and superphosphate or rock- phosphate, contrary to plants grown in soil unamended with phosphatic fertilizer.VAMF can be considered one tactic to be included in an IPM program provided enough phosphatic sources are available.

INTRODUCTION

The role of arbuscular mycorrhiza fungi (AMF) in the control of plant pathogens has been the subject of several reviews (Dehne, 1982; Sharma *et al.*,1992). AMF were often reported to increase plant resistance against soil-borne diseases, whereas shoot and foliar diseases have rather increased in severity following mycorrhizal inoculation. Liu (1995) reported that the incidence Verticillium wilt of cotton was reduced by the inoculation with VAMF. Liu *et al.* (1995) also found that the increased disease resistance of plants colonized by VAMF is mostly due to the increased availability and the uptake of phosphorus.

The objective of the present study was to investigate the effect of VAMF and P on the severity of infection by *Fusarium oxysporum, Verticillium albo-atrum* and *Colletotrichum trifolii* in alfalfa plants.

MATERIALS AND METHODS

Isolation and identification of VA Mycorrhiza spores were made following the method reported by Daniels and Skipper (1991).

Soil samples were collected from fields under different crops. A 100 gm soil sample was first stirred thoroughly in 5L water and then allowed to settle for 15sec. The contents were then decanted through 0.5 mm sieve into a second container, in which the suspension was swirled vigorously and again allowed to settle for 15sec. The supernatant was poured through a 0.036 mm sieve and the trapped material was washed into a beaker. After stirring, the suspension was centrifuged for 4 minutes at 3000 rpm. The supernatant was replaced by a 50% sucrose solution and the pillet was resuspended and then centrifuged for 15sec at 3000 rpm. The resulting supernatant was passed through a 0.036 mm sieve and finally, the residue remaining on the sieve was washed with water to remove the sucrose solution. The residue was washed into a Petri dish and examined under a binuclear and also under a microscope.

Mycorrhizal fungi were identified at Dept. of microbiology, Fac. Agric., Suez Canal Univ., according to Gerdeman and Trappe (1974) and identification was confirmed by M. Vestberg at the Agric. Res. Station, Lukka, Finland.

Plant pathogenic fungi:

All pathogenic fungi used were isolated and maintained at the Dept. plant pathology, Ismailia Agric. Res. Station (IARS) by El-Barougy. These isolations were made during surveys conducted within the activity of Egypt-Finland Agric. Res. Project.

Greenhouse experiments :

Role of endomycorrhiza in the control of some diseases of alfalfa in the presence or absence of phosphorus supplements:

A pot experiment was conducted using 30 cm pots filled with non-cultivated sandy soil. Pots were infested separately with the pathogenic fungi (*Fusarium oxysporum*, *Verticillium albo-atrum* and *Colletotrichum trifolii)*

Fungal inocula were prepared by growing each fungus in potato dextrose (PD)

liquid medium in flasks (500 ml), incubated at 27°C for 15 days. The fungal growth was added, after being homogenized in a Waring blender to potted soil at the rate of 50 ml per pot and thoroughly mixed.

The pots infested with a given fungus, were divided into sub-sets. One sub-set was amended with superphosphate (15.5 % P_2O_5) at the rate of 3.3 gm / pot the second was amended with rock phosphate (27% P_2O_5) at the rate of 1.87 gm / pot and the third was left without phosphate amendment to serve as a control. Such rates are equivalent to 30kg/ feddan.

A mycorrhizal inoculum containing the three identified genera *Glomus*, *Gigaspora* and *Acculospora* was prepared by embedding in water agar in Petri dishes. The agar culture in every Petri dish was divided into small pieces ($1cm^2$),two of which were used to inoculate the subsets of phosphate treatments (60 chlamydospores) and the other was left without treatment, consequently six treatments were obtained.

Each pot was sown with 10 seeds of cv. Sewa of alfalfa provided by the Dept. crop production at the IARS. Samples of the plants were taken after 14, 45, and 90 days. At each growth stage, shoots and roots weights and percentage of infection with pathogenic fungi were determined.

Mycorrhizal colonization in alfalfa roots was determined according to the method reported by Phillips and Hayman (1970). Randomly selected segments of fine, lateral roots were mounted on microscopic slides and examined to assess the proportion of root length with mycorrhizal colonization. The presence of vesicles, arbscules and any unusual features were recorded. Degree of colonization was made as proposed by Giovannetti and Mosse (1980).

- = None + = 2 - 25% ++ = 25 - 50 % +++ = 50 - 75 % ++++ = 75 - 100 %.

Soil available phosphorus was extracted with sodium bicarbonate and determined according to the method of Olsen *et al.* (1954).

EXPERIMENTAL RESULTS

Isolation and identification of Vesicular- Arbuscular Mycorrhiza fungi (VAM):

VA mycorrhiza fungi were found in soil samples collected from different locations of Ismailia governorate. The isolates were identified as *Glomus* sp, *Gigaspora* sp, *Acalospora* sp; however, most of the identified isolates belonged to the genus *Glomus* (Fig.1), according to Gerdeman and Trappe (1974).

Effect of V.A. mycorrhiza on the incidence of alfalfa diseases in the presence of different phosphorus sources:

A. Fusarium damping-off:

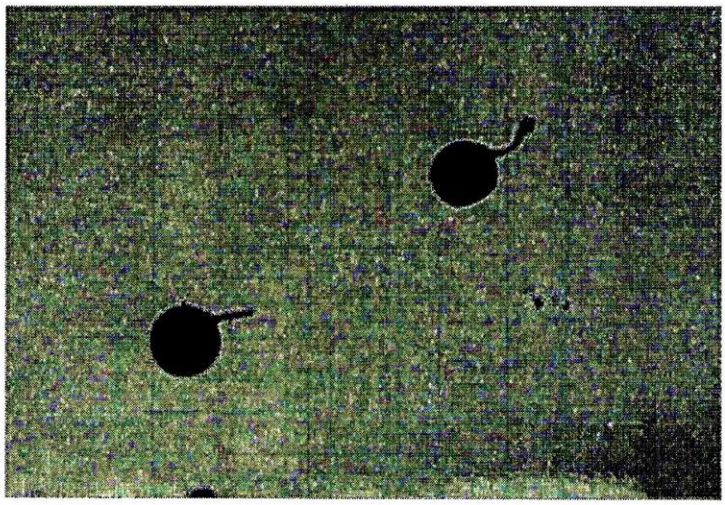
Data presented in Table (1) show that the severity of Fusarium infection decreased in plants grown in soil amended with mycorrhiza and superphosphate or rock-phosphate, reaching 23.3, 26.6% respectively, while it was not negatively affected when mycorrhiza was found in soil unamended with phosphate sources where infection reached 40%.(Fig. 2, 3).

The extent of mycorrhizal colonization of alfalfa roots increased with the addition of superphosphate to the soil (50-100%) as compared to rock phosphate or in the absence of supplemental phosphatic fertilizer (2-25%). This was the case 45 and 90 days after planting.

The available phosphorus in soil was affected by the type of phosphatic material applied, where it was much higher (115ppm) with superphosphate compared with rock phosphate (23.3ppm) 15 days after planting. A sharp decrease in the amount of available phosphorus in the soil was noticed as the plants grew older and to a greater extent in the presence of mycorrhiza and superphosphate. After 90 days, the available P reached 1 and 3 ppm in case of fertilization with superphosphate & rock phosphate, respectively.

Dry weights of plants were affected by VA mycorrhiza and phosphate fertilization as well as the age of the plant. They increased in presence of mycorrhiza with or without phosphatic fertilization in comparison with soil devoid of mycorrhiza during all stages. However, dry weights were lower for plants growing in soil unamended with

A



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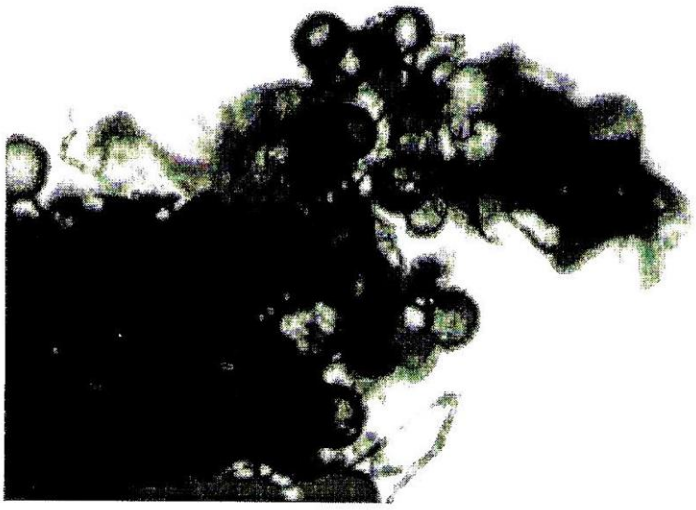


Fig. 1. Chlamydospores of Glomus sp-arbuscular mycorrhizal fungus.
A. A single chlamydospore and hyphal attachment x 100.
B. Glomus sp. (sporocarp) x 100.

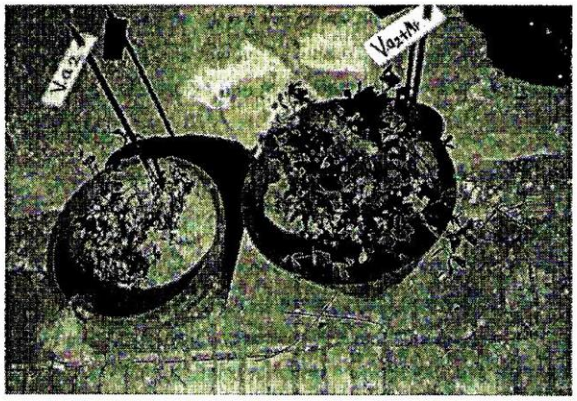
Table 1. Percentage of Fusarium infection , shoot dry weight of plants and available phosphate in soil in response to mycorrhizal infestation and phosphatic amendments.

Treatment	dry shoot weight,available phosphate and degree of colonization																											Percentage of *F.oxysporium* infection		
	15 days after planting									45 days after planting									90 days after planting											
	dry shoot weight g/plant			Available Phosphate (ppm)			dgeree of colonization*			dry shoot weight g/plant			Available Phosphate (ppm)			dgeree of colonization*			dry shoot weight g/plant			Available Phosphate (ppm)			dgeree of colonization*					
	Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Soil infested with VAM	0.64	0.2	0.35	115.1	23.3	16.4	-	-	-	5.8	0.87	0.38	10.9	5.4	4.39	+++	+	+	7.12	1.9	1.5	1	3	8	++++	+	+	23.3	26.6	26.7
Soil uninfested with VAM	0.35	0.12	0.24	104.5	6.8	9.6	-	-	-	2.06	0.42	0.5	14.2	2.1	6.5	-	-	-	3.6	1.7	1.01	0.5	3	9	-	-	-	40	40	26.7

(A) soil amended with superphosphate (B) soil amended with rock-phosphate (C) Control without fertilizer

*according to (Goivannetti and Mosse, 1980) - = None + = 2- 25%

++ = 25 – 50 % +++ = 50 – 75% ++++ = 75 – 100%



A B

Fig 2. Effect of (VAM) on the control of *V.albo-atraum.*

A. Infested soil with *V.albo-atraum* 45 days after planing.

B. Infested soil with *V.albo-atraum* + VAM 45 days after planing.

A B

Fig 3. Effect of (VAM) on the control of *F.oxysporum.*

A. Infested soil with *F.oxysporum* 45 days after planing.

B. Infested soil with *F.oxysporum* + VAM 45 days after planing.

phosphatic fertilizer. Also, the increase in dry weight with age was always seen in the absence of mycorrhiza reaching 3.6 and 1.7 g/plant at 90 days with super- and rock phosphate, respectively compared with 7.12g and 1.90g in the presence of mycorrhiza.

B. Verticillium infection

Data in Table (2) show that the percentages of Verticillium infection decreased when plants were treated with mycorrhiza and fertilized with superphosphate or rock phosphate showing 13.3 and 23.3% respectively compared with 33.3 in the unfertilized control. Infection increased in soil unamended with phosphate even in the presence of mycorrhiza and infection was higher in plants not colonized by mycorrhiza regardless of phosphate fertilization.

The extent of mycorrhizal colonization was affected by the presence of phosphorus as well as the age of plants. Mycorrhizal colonization increased by using superphosphate during the first 45 day after planting to reach 100%; however, the effect of rock phosphate in increasing mycorrhizal colonization to 100% was delayed to 90 day after planting .

The highest values of available phosphorus were detected when plants treated with mycorrhiza were grown in soil amended with superphosphate compared to rock phosphate or without fertilizer in all stages.

Available phosphorus in soil decreased after 45 day old compared to the first stage (15 days) from 131.6 ppm to 21.9 with superphosphate and from 8.2 to 4.3 with rock phosphate. At the sametime, the available phosphate decreased in mycorrhizal treatments during the first 45 days as compared to non -mycorrhizal treatments.

Dry weight of shoots increased in plants grown in mycorrhiza- treated soil amended with super or rock- phosphate (5.3 and o.85g respectively), whereas in soil unamended with phosphatic fertilizer, dry weight decreased in case of mycorrhiza treatment during 45 days following planting (0.34g /plant).

Table 2. Percentage of Verticillium infection , shoot dry weight of alfalfa plants grown in soil infested and noninfested with Vesicular Arbscular Mycorrhiza (VAM) and *V.albo-atrum* and amended with and without superphosphate or rock-phosphate, and the available phosphorus in soil at different intervals.

Treatment	dry shoot weight,available phosphate and degree of colonization																									Percentage of *V. albo atrum* infection		
	15 days after planting									45 days after planting									90 days after planting									
	dry shoot weight g/plant			Available Phosphate (ppm)			dgeree of colonization*			dry shoot weight g/plant			Available Phosphate (ppm)			dgeree of colonization*			dry shoot weight g/plant			Available Phosphate (ppm)			dgeree of colonization*					
	Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Soil infested with VAM	0.37	0.19	0.21	131.6	8.2	13.7	-	-	-	5.3	0.85	0.34	21.9	4.3	3.2	++++	+++	+++	7.68	0.9	1.5	15	2	12	++	++++	+++	13.3	23.3	33.3
Soil uninfested with VAM	0.30	0.12	0.19	95.9	13.7	6.3	-	-	-	2.6	0.63	0..5	24.1	6.5	5.4	-	-	-	4.06	0.9	1.3	4	2	14	-	-	-	26.6	33.3	26.7

(A) soil amended with superphosphate (B) soil amended with rock-phosphate (C) Control without fertilizer

* according to Goivannetti and Mosse (1980) - = None + = 2- 25% ++ = 25 – 50 % +++ = 50 – 75% ++++ = 75 – 100%

Colletotrichum damping-off :

As shown in Table (3) plants growing with VA mycorrhiza showed a reduction in the percentages of *C. trifolii* damping off in soil amended with either super or rock phosphate at 30 and 40% respectively compared with 46.6% in un-fertilized treatment. In the absence of mycorrhizal infestation disease percentages were 43.3, 53.3 and 40% with superphosphate, rockphosphate and no phosphate, respectively.

Mycorrhizal root colonization was higher in soil amended with rock phosphate and in that receiving no phosphatic fertilizers compared with that in plants growing in soil amended with superphosphate 45 and 90 days after planting.

Concentration of available phosphorus in soil were lower wherever VAM was present compared with its level in the absence of mycorrhiza and which has dropped drastically by time. Available P was always higher at 15 days with superphosphate treatment compared with rock phosphate.

DISCUSSION

Mycorrhizal fungi are a group of fungi which establish a special relationship with certain plant species. This relationship has multiple and diverse effects on the colonized plant species. Some of these effects have been implicated in the process of protection against some root- infecting fungi. Mycorrhizal fungi were isolated and identified as *Glomus* sp, *Gigaspor* sp and *Acalospora* sp. However, most of the identified isolates in the present study belonged to the genus *Glomus*. Stahl and Christensen (1982) stated that *Glomus* sp has been reported as the most common arbuscular mycorrhizal fungi (AMF) predominating in the temperate regions (Gerdeman and Trappe, 1974; Miller *et al.*, 1985). In an attempt to control damping off disease in alfalfa (sewa cv.) ,the study was carried out using VA mycorrhiza with or without different phosphate sources added to the planting soil. Much of the literature suggests that VAM fungi reduce the incidence of soilborne diseases and/ or the effect of the resulting disease caused by fungal pathogens. Dehne and Schonbeck (1979) showed that VAM reduced Fusarium wilt (*F.oxysporum f. sp lycopersici)* of tomato, Becher (1976) reported a similar effect on pink root of onion. Vidhyasekaran (1990) recorded many instances of controlling root diseases with VAM fungi and Sharma *et al.* (1992) showed that arbuscular mycorrhizal

Table 3. Damping off, shoot dry weight of alfalfa plants grown in soil infested with vesicular arbuscular mycorrhiza (VAM) and *C.trifolii* and amended with superphosphate or rock-phosphate and the available soil phosphorus after different intervals.

Treatment	dry shoot weight,available phosphate and degree of colonization																											Percentage of *C.trifolii* damping off		
	15 days after planting									45 days after planting									90 days after planting											
	dry shoot weight g/plant			Available Phosphate (ppm)			dgeree of colonization*			dry shoot weight g/plant			Available Phosphate (ppm)			dgeree of colonization*			dry shoot weight g/plant			Available Phosphate (ppm)			dgeree of colonization*					
	Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer			Fertilizer		
	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C	A	B	C
Soil infested with VAM	0.47	0.3	0.5	115.1	8.6	12.3	-	-	-	2.56	0.75	0.37	18.1	5.5	8.7	+	++	++	4.48	1.7	1.15	1	1	9	+	+++	+	30	40	46.6
Soil uninfested with VAM	0.41	0.15	0.39	104.5	12.3	8.3	-	-	-	2.12	0.66	0.92	17.5	2.2	5.48	-	-	-	4.08	1.1	2.28	9	15	9	-	-	-	43.3	53.3	40

(A) soil amended with superphosphate (B) soil amended with rock-phosphate (C) Control without fertilizer

* according to (Goivannetti and Mosse, 1980) - = None + = 2- 25%

++ = 25 – 50 % +++ = 50 – 75% ++++ = 75 – 100%

fungi (AMF) infection, generally, protects plants against soil-borne fungi.

The results reported herein showed that VA mycorrhiza decreased the severity of Fusarium damping off in plants grown in soil amended with superphoste or rock phosphate, while Fusarium infection increased in absence of mycorrhiza in soil amended with phosphate source. Similar results were observed with both *V. albo-atrum* and *C.trifolii.* Recent literature, indicates that the biological control of plant diseases may, in some of VAM cases, be due to improved nutrition. The most obvious contribution of VAM is the increase in nutrient uptake particularly P and other minerals, resulting in more vigorous plants better able to resist or tolerate root infection. The evidence supporting the enhanced nutrition idea comes from experiments where effects comparable to VAM were observed when more P fertilizer was added. Davies (1980) showed this type of response in his studies on Thielaviopsis root rot of citrus where VAM plants were larger than non VAM plants unless the latter were fertilized with P. Similar effect on Phytophthora root rot of citrus was also recorded by Davies and Menge (1980)

The extent of mycorrhizal colonization increased with the addition of superphosphate to the soil in comparison with rock phosphate or in case of no phosphate added and that mycorrhizal colonization was not affected by plant age (45-90 day) in case of infestation with *Verticillium albo-atrum.* Mycorrhizal colonization was affected by treating plants with phosphate sources and age of plants. Colonization increased by using superphosphate during the time interval 15-45 days after planting, but it increased with rock phosphate during 45-90 days interval. Role of VAM in biological control of *Colletotrichum* damping off was observed. The extent of mycorrhizal colonization of plants grown in soil treated with rock phosphate or un-treated with phosphatic fertilizer was higher than in soil amended with superphosphate at 45 and 90 days.

Dry weight of plant was affected by VA mycorrhiza and phosphatic fertilization, where it increased in the presence of mycorrhiza and superphosphate but was lower when soil was infested with VAM and unamended with phosphatic fertilizers. Khan (1972) mentioned that, in field experiments,maize showed great increases in shoot dry matter as a result of inoculation with vesicular - arbuscular mycorrhiza . Also, Plenchette *et al* (1982) found that VA mycorrhizal inoculation of strawberry plants increased the number of flowers and fruits, and sometimes vegetative growth. Ishac *et al* (1993)

demonstrated that the growth of faba bean occurs in calcareous desert soil by inoculating rhizobia and VA mycorrhizae and the addition of rock or superphosphate.

It is apparent from the present study that mycorrhiza plays a positive role in the nutrition of the plant and can be considered as one important element in an integrated program for protecting roots against certain root infecting fungi.

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دور الميكورهيزا الداخلية في مقاومة بعض المسببات المرضيه فى البرسيم الحجازى

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تتعرض نباتات البرسيم الحجازي للاصابة بالعديد من الفطريات وتعتبر أمراض الذبول واعفان الجذور و التيجان التى تصيب المجموع الجذرى و المتسببة عن فطريات الفيوزاريم أوكسيسبوريم والفيرتيسيليوم ألبواترم و الكوليتوتريكم من الامراض الهامه وقد تم دراسة دور الميكرهيزا فى مقاومة هذه الامراض.

فقد تم عزل و تعريف فطريات الميكورهيزا الداخلية من التربة المزروعة بمحاصيل مختلفة و كانت الفطريات المعزولة معظمها من جنس جلومس *Glomus* والقليل منها من جنس *Gigaspora* و جنس *Acalospora*.

تم دراسة دور الميكورهيزا الداخلية فى المقاومة البيولوجية فى وجود مصادر مختلفة من الفوسفور مثل السوبر فوسفات و صخر الفوسفات فى تربة محقونة بفطريات الفيوزايم و الفيرتسيليم و الكوليتوتريكم ؛ و قد لوحظ ان استخدام الميكورهيزا الداخلية مع وجود مصدر للفوسفات (سوبر فوسفات او صخر الفوسفات) يعمل على تقليل نسبة الذبول في النباتات المزروعة في التربة التى لوثت ا بالفطريات السابقة. كما ادى استخدام الميكورهيزا مع عدم استخدام مصدر للفسفور الي زيادة نسبة موت البادرات.كما زادت درجة استعمار الميكروهيزا للنبات بإضافة السوبر فوسفات إلى التربة مقارنة بكل من إضافة صخر الفوسفات أو عدم استخدام سماد فوسفاتي و قد تأثر الوزن الجاف للمجموع الخضري باستخدام الميكروهيزا مع التسميد الفوسفاتي حيث زاد الوزن الجاف في وجود الميكروهيزا مع السوبر فوسفات أو صخر الفوسفات بينما نقص الوزن الجاف للمجموع الخضري باستخدام الميكروهيزا في التربة الغير معاملة بالأسمدة الفوسفاتية.

ويمكن اعتبار الميكورهيزا أحد الوسائل التى تدخل فى برنامج متكامل لمكافحة بعض الامراض التى تصيب الجذور. شريطة توفير قدر كافي من التسميد الفوسفاتي.