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INOCULATION WITH SOIL ARBUSCULAR MYCORRHIZAL FUNGI AND PHOSPHATE-SOLUBILIZING BACTERIA AS A MEANS FOR IMPROVING ROCK PHOSPHATE AVAILABILITY FOR THOMPSON SEEDLESS GRAPE ROOTINGS

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ABSTRACT

This study was carried out to disclose the effect of inoculation with arbuscular mycorrhizal fungi and phosphate solubilizing bacteria on the soil fertilized with two rates of rock phosphate material (50 or 100 mg rock phosphate/kg soil) in an attempt improve vegetative growth, nutritional status and microbiological and enzyme activity in the rhizosphere of Thompson Seedless grape rootings through two successive seasons (2006 & 2007).

The interaction between arbuscular mycorrhizal fungi and phosphate solubilizing bacteria at one hundred mg of rock phosphate fertilizer gave the best results where it was accompanied by improving vegetative growth: shoot length, shoot diameter, number of leaves/rooting, leaf area, coefficient of wood ripening, shoot and root biomass, total biomass and root/shoot ratio. Total chlorophyll and percentages of total nitrogen, phosphorus and potassium of the leaves and total carbohydrates of shoots were substantially increased. Concerning the microbiological and enzyme activity in the rhizosphere of Thompson Seedless grape rootings, it was noticed that the percentage of infection of AM-micorrhizae, spore numbers of AM-micorrhizae, total count of phosphate solubilizing bacteria, populations of total microorganism, dehydrogenase and phosphatase enzyme activity in the rhizosphere were also increased.

INTRODUCTION

Plant mineral nutrition depends mainly on the phosphorus content of soil, which can be assimilated only as soluble phosphate. Hence the use of rock phosphate as a fertilizer for phosphorus deficient soils has received a significant interest in the recent years since they are natural, inexpensive and available fertilizers. However their solubilization rarely occurs in alkaline soils (Caravaca *et al.*, 2005). Physical and chemical weathering of rock phosphate is mainly realized along plant roots in the rhizosphere. This part of soil supports large microbial communities that facilitate weathering of minerals by several mechanisms such as lowering of pH by producing organic acids, phenolic compounds, siderophores, ion chelation and exchange reactions in the growth environment which have been reported to play a role in phosphate solubilization by phosphate solubilizing microorganisms (Drever and Vance, 1994). Numerous studies identified microbial groups which could solubilize mineral phosphates and improve plant phosphorus nutrition (Gadd, 1999). Among microbial groups that can solubilize mineral phosphates, allowing the sustainable use of phosphate fertilizers and improving plant phosphorus

nutrition are AM fungi and bacteria (Asea *et al.*, 1988 and Singal, *et al.*, 1994).

Aarbuscular mycorrhizal (AM) fungi have been found to be essential components of sustainable soil-plant systems (Van der Hejden *et al.*, 1998 and Schreiner *et al.*, 2003). They increased plant uptake of phosphorus (Duponnois *et al.*, 2005), nitrogen (Barea *et al.*, 1991) and micronutrients (Burkert and Robson, 1994). VA mycorrhizal fungi are capable of dissolving weakly soluble soil minerals by releasing acids (Leyval, and Berthelin, 1989) or increasing CO_2 partial pressure (Knight, *et al.*, 1989). Therefore, they have the ability to enhance host plant uptake of relatively immobile nutrients particularly phosphorus (Thompson, 1987). Mycorrhizal inoculation improves the absorption of phosphorus and other nutrients (Clark and Zeto 2000) and possibly facilitating nutrient transport among plants (Chen *et al.*, 2005). The main explanation is that mycorrhizas developed an extramatrical mycelium which increased the root phosphorus absorbing sites (Bolan, 1991). This mycorrhizal effect has been frequently observed in rock phosphate amended soils and it induced spectacular stimulation of the microbial activity, nutrient uptake and plant growth in the rhizosphere by increasing plant height and dry matter production of root and shoot. (Fay *et al.*, 1996; Techapinyawat *et al.*, 2000; Caravaca *et al.*, 2005; Duponnois *et al.*, 2005 and Raja 2006).

The inoculation of phosphate-solubilizing bacteria is a promising technique as it can increase phosphorus availability in soils fertilized with rock phosphates (Reyes *et al.*, 2002). There are several indications that bacteria have important functions in the interaction between roots and arbuscular mycorrhizal fungi (Fester *et al.*, 1999). The phosphate-solubilizing rhizobacteria behaved as mycorrhiza helper bacteria (Garbaye, 1994) since they promoted root colonization when associated with mycorrhizal fungi, confirming the previous findings involving other AM fungi and phosphate solubilizing bacteria combinations (Azcon-Aguilar, *et al.*, 1986).

The interaction of phosphate-solubilizing bacteria and arbuscular mycorrhizal fungi can promote better establishment of arbuscular mycorrhizal fungi by phosphorus ions released from rock phosphate which are taken up by the AM mycelium, thereby maintaining a low soluble phosphorus concentration in the discrete soil microhabitats where the rock phosphate particles are attacked by the phosphate-solubilizing bacteria and thus favoring a continuous phosphorus release (Toro *et al.*, 1997). The interaction between AM fungi and phosphate-solubilizing bacteria contributed to the biogeochemical cycling of nutrients by more than just providing a greater surface area for scavenging mineral nutrients that may be relatively immobile in the soil or in a short supply (Linderman and Pfleger 1994).

The principal objective of this study is to evaluate solubilization ability of rock phosphate through the inoculation with arbuscular mycorrhizal fungi and phosphate solubilizing bacteria in Thompson Seedless grape rootings.

MATERIALS AND METHODS

This study was conducted during 2006 and 2007 seasons in the shade house of the Horticultural Research Institute, Giza, Egypt. Three hundred and

sixty uniform and healthy one-year-old own rooted Thompson Seedless grape rootings were selected. The rootings were planted in the first week of March in polyethylene bags with a capacity of five kgs and were grown in a sandy loam soil. Physical, chemical and microbiological analysis were as follows: sand (74.2%), silt (3.1 %), clay (22.7%), organic carbon (0.05%), pH (7.60), EC (1.40Mmhos/cm), water holding capacity (23.0%), Ca Co3 (0.7%), N (1.13%), P (0.09%), K (0.59%), number of AM (3.1spore/g soil), AM infection (5.7%) and total microbial count (10.1 x 10^5cfu/g soil). All bags had bottom holes to allow excess the drainage of water.

The applied treatments were as follows:

1. Control (Untreated plants).
2. Fertilizing with rock phosphate at 50 mg/1 kg soil (50 mg RP).
3. Fertilizing with rock phosphate at 100 mg/1 kg soil (100 mg RP).
4. Inoculation with arbuscular mycorrhizal fungi (AM).
5. Inoculation with phosphate solubilizing bacteria (PSB).
6. (AM) + (PSB).
7. (50 mg RP) + (AM).
8. (100 mg RP) + (AM).
9. (50 mg RP) + (PSB).
10. (100 mg RP) + (PSB).
11. (50 mg RP) + (AM) + (PSB).
12. (100 mg RP) + (AM) + (PSB).

Each treatment was replicated three times, each ten plants acted as a replicate (i.e. 30 plants for each treatment).

Phosphate Fertilizers: rock phosphate (24.5% P_2O_5) was purchased from Al-Ahram Company for Natural fertilizers, Giza, Egypt. Two rates of this rock material (50 or 100 mg/1 kg soil) were applied in the form of finely (100-mesh) ground natural product.

Mycorrhizal spores were originally extracted from the Egyptian soil. Spores of AM-mycorrhizae including the following genera Glomus, Gigaspora and Acaulospora were added after planting. Extraction and counting of identified mycorrhizal spores were carried out according to the method described by (Massoud, 1999). Fifty grams per pot of mixed spores (250 spores/gram) of AM-mycorrhizal genera were prepared after extraction and the roots plants of each pot were immersed in sugary solution then coated with micorrhizal spores carried on sterilized sand soil as a carrier modified by (Massoud 2005).

Phosphate solubilizing bacteria (Bacillus megaterium) were grown on a medium (Bunt and Rovira 1955) and enriched on nutrient growth medium (Difco 1984). It was added to the soil at the rate of 1 ml /1 kg soil (10^9 cfu) after planting.

The following parameters were estimated at the end of each experimental season:

1. Morphological studies:

- Vegetative growth parameters

Shoot length (cm), shoot diameter (cm), number of leaves/plant, average leaf area (cm^2) of the apical 5^{th} and 6^{th} leaves using a CI-203- Laser Area-meter made by CID, Inc., Vancouver, USA. were recorded in both

seasons. Total leaf area/plant (cm^2) was determined by multiplying total number of leaves per plant by average leaf area. Coefficient of wood ripening was calculated by dividing length of the ripened part of the shoot by total length of the shoot according to Bouard (1966).

- Plant biomass

Shoot biomass (g dry weight), root biomass (g dry weight). total biomass (g dry weight) and root/shoot ratio were recorded.

2. Chemical studies:

- Leaf content of total chlorophyll. This was measured by using nondestructive Minolta chlorophyll meter SPAD 502 (Wood *et al.*, 1992).
- Leaf content of total nitrogen (%) (Pregl, 1945), phosphorus (%) (Snell and Snell 1967) and potassium (%) (Jackson, 1967).
- Shoot content of total carbohydrates (%) (Smith *et al.*, 1956).

3. Microbiological studies:-

Samples were taken for carrying out the following determinations:

1- AM infection (%): estimated according to (Massoud, 2005).
2-Number of AM (spore/g soil): estimated according to (Massoud, 2005).
3- Total count of phosphate solubilizing bacteria (-x10^5 cfu/g soil).
4-Total microbial count (-x10^5 colony forming unit (cfu)/g soil) estimated according to (Esher and Jensen 1972).
5-Dehydrogenase enzyme activity (µgTPF/g/D.W.soil/day) estimated according to Salman (1967).
6-Phosphatase enzyme activity (IP/g/D.W.soil/day) estimated according to Drobnikova (1961).

*** Statistical analysis:**

The complete randomized design was adopted for the experiment. The statistical analysis of the present data was carried out according to Snedecor and Chocran (1980). Averages were compared using the new L.S.D. values at 5% level.

RESULTS AND DISCUSSION

1. Morphological studies:

- Vegetative growth parameters

Data shown in (Table 1) revealed that all vegetative growth parameters ; i.e. shoot length, number of leaves, shoot diameter, total leaf area and coefficient of wood ripening) responded positively to arbuscular mycorrhizal fungi (AMF) and/or phosphate solubilizing bacteria (PSB) inoculation at different tested doses of the rock phosphate amendment. The highest values of these estimated were detected in case of plants treated with the combined inoculation of AMF and PSB at one hundred mg of rock phosphate amendment followed by the combined inoculation of AMF and PSB at fifty mg of rock phosphate amendment compared to control in both seasons of the study.

- Plant biomass

The results concerning dry biomass production in Thompson Seedless grape plants in response to AM and PSB inoculations and rock phosphate amendment are presented in (Table, 2).

Table (1): Effect of inoculation with arbuscular mycorrhiza (AM) fungi and phosphate solubilizing bacteria in rock phosphate amended soil on some vegetative growth characteristics of Thompson Seedless grape rootings (2006 and 2007 seasons)

Caracteristic / Treatment	Average shoot length (cm)		Average shoot diameter (cm)		No. of leaves/shoot		Average leaf area/shoot (cm^2)		Total leaf area/plant (cm^2)		Coefficient of wood ripening	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Control	42.2	45.4	0.69	0.72	16.2	19.2	94.0	98.5	1522.80	1891.20	0.72	0.79
50 mg RP	43.6	45.7	0.69	0.72	16.7	19.5	94.4	100.4	1576.48	1957.80	0.72	0.80
100 mg RP	47.5	47.9	0.71	0.77	19.1	20.6	101.0	107.3	1929.10	2210.38	0.74	0.84
AM	47.8	48.0	0.72	0.77	19.6	20.8	104.0	109.6	2038.40	2279.68	0.75	0.85
PSB	45.2	46.9	0.70	0.74	17.6	19.9	97.3	102.7	1712.48	2043.73	0.73	0.82
AM + PSB	49.0	48.6	0.73	0.78	21.9	21.6	107.8	112.8	2360.82	2436.48	0.75	0.86
50 mg RP + AM	49.5	52.6	0.78	0.80	22.7	24.2	112.5	118.2	2553.75	2860.44	0.80	0.87
100 mg RP + AM	50.5	53.7	0.79	0.83	24.2	25.1	112.6	118.7	2724.92	2979.37	0.80	0.87
50 mg RP + PSB	49.2	49.9	0.75	0.79	22.0	22.0	108.9	115.2	2395.80	2534.40	0.77	0.86
100 mg RP + PSB	49.4	52.6	0.77	0.80	22.4	24.1	111.1	117.3	2488.64	2826.93	0.80	0.86
50 mg RP + AM + PSB	50.9	54.9	0.80	0.84	26.0	26.5	114.3	120.8	2971.80	3201.20	0.82	0.87
100 mg RP + AM + PSB	51.2	57.1	0.81	0.84	26.5	27.8	116.3	121.1	3081.95	3366.58	0.86	0.89
new L.S.D. at 0.05 =	3.8	4.1	0.05	0.07	6.3	6.7	12.3	12.9	717.6	728.9	0.07	0.09

Table (2): Effect of inoculation with arbuscular mycorrhiza (AM) fungi and phosphate solubilizing bacteria in rock phosphate amended soil on shoot biomass, root biomass, total biomass and root/shoot ratio of Thompson Seedless grape rootings (2006 and 2007 seasons)

Caracteristic / Treatment	Shoot biomass (g dry weight)		Root biomass (g dry weight)		Total biomass (g dry weight)		Root/shoot ratio	
	2006	2007	2006	2007	2006	2007	2006	2007
Control	4.92	5.37	8.14	8.57	13.06	13.94	1.65	1.60
50 mg RP	6.09	6.50	9.13	9.84	15.22	16.34	1.50	1.51
100 mg RP	6.87	7.06	10.52	10.91	17.39	17.97	1.53	1.55
AM	7.28	7.39	11.43	11.97	18.71	19.36	1.57	1.62
PSB	6.06	6.57	10.37	11.23	16.43	17.80	1.71	1.71
AM + PSB	7.36	7.53	12.92	13.46	20.28	20.99	1.76	1.79
50 mg RP + AM	7.45	7.76	14.06	14.47	21.51	22.23	1.89	1.86
100 mg RP + AM	7.64	7.93	15.23	15.91	22.87	23.84	1.99	2.01
50 mg RP + PSB	6.61	6.84	13.07	13.69	19.68	20.53	1.98	2.00
100 mg RP + PSB	6.97	7.14	14.21	14.68	21.18	21.82	2.04	2.06
50 mg RP + AM + PSB	8.07	8.39	16.83	17.59	24.90	25.98	2.09	2.10
100 mg RP + AM + PSB	8.85	9.02	18.86	19.36	27.71	28.38	2.13	2.15
new L.S.D. at 0.05 =	0.52	0.63	2.11	2.27	2.49	2.61	0.17	0.19

All AM inoculation treatments either applied alone or in combination with PSB at different doses of rock phosphate amendment significantly surpassed non-inoculated (control). The combined inoculation of AM and PSB at one hundred mg of rock phosphate recorded the highest values of shoot biomass (g dry weight), root biomass (g dry weight), total biomass (g dry weight), root/shoot ratio compared to non-inoculated and unamended treatment (control) in both seasons of the investigation followed by the combined inoculation of AM and PSB at fifty mg of rock phosphate amended.

The positive effect of AM and PSB inoculations and rock phosphate amendment on vegetative growth parameters could be explained by that mycorrhizal inoculation improves the absorption of phosphorus and other nutrients (Clark and Zeto 2000) and possibly facilitating nutrient transport among plants (Chen *et al.*, 2005). The main explanation is that mycorrhizas developed an extramatrical mycelium which increased the root phosphorus absorbing sites (Bolan, 1991). This mycorrhizal effect has been frequently observed in rock phosphate amended soils and it induced spectacular stimulation of the microbial activity, nutrient uptake and plant growth in the rhizosphere by increasing plant height and dry matter production of root and shoot. (Fay *et al.*, 1996; Techapinyawat *et al.*, 2000; Caravaca *et al.*, 2005; Duponnois *et al.*, 2005 and Raja 2006). Moreover, the phosphate-solubilizing rhizobacteria behaved as mycorrhiza helper bacteria (Garbaye, 1994) as they promoted root colonization when associated with mycorrhizal fungi, confirming the previous findings involving other AM fungi and phosphate solubilizing bacteria combinations (Azcon-Aguilar, *et al.*, 1986).

2. Chemical studies:

Inoculation with arbuscular mycorrhizal fungi (AMF) and/or phosphate solubilizing bacteria (PSB) inoculations at different tested doses of the rock phosphate amendment improved the chemical characteristics of vegetative growth (Table, 3).

- Leaf content of total chlorophyll

The effect of the conducted treatments was significantly evident on leaf content of total chlorophyll. Combined inoculation of AM and PSB at one hundred mg of rock phosphate amendment resulted in the highest values of total chlorophyll as compared to non-inoculated and unamended treatment (control) in both seasons.

The results are in agreement with those obtained by (Krishna *et al.*, 2005) who reported that the mycorrhizal inoculation of micropropagated grape plantlets significantly enhanced chlorophyll content in leaves. The enhanced chlorophyll level might he responsible for increasing photosynthesis in inoculated plantlets as observed in the present study. This can further be attributed to increased Mg and Fe uptake, which are essential for chlorophyll bio-synthesis.

- Leaf content of total nitrogen, phosphorus and potassium

As for the nutrient acquisition i.e. percentages of total nitrogen, phosphorus and potassium of the leaves, it can be noticed that all AM inoculation treatments either solely or in combination with PSB inoculation at different doses of rock phosphate amendment showed a pronouncing effect on nutrient acquisition.

Table (3): Effect of inoculation with arbuscular mycorrhiza (AM) fungi and phosphate solubilizing bacteria in rock phosphate amended soil on leaf content of total chlorophyll, N, P, K and shoot content of total carbohydrates of Thompson Seedless grape rootings (2006 and 2007 seasons)

Caracteristic / Treatment	Total chlorophyll (mg/g F.W.)		N (%)		P (%)		K (%)		Total carbohydrates (%)	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Control	30.9	23.8	0.47	0.46	0.18	0.23	0.38	0.44	20.1	21.2
50 mg RP	32.3	24.1	0.49	0.48	0.21	0.25	0.39	0.45	20.5	21.5
100 mg RP	36.2	26.3	0.51	0.49	0.26	0.29	0.42	0.45	21.2	22.5
AM	36.5	26.4	0.52	0.51	0.28	0.31	0.43	0.47	22.1	23.3
PSB	33.9	25.3	0.49	0.49	0.25	0.28	0.41	0.45	20.9	22.3
AM + PSB	37.7	27.0	0.53	0.53	0.29	0.31	0.44	0.47	22.5	23.8
50 mg RP + AM	38.2	31.0	0.57	0.60	0.32	0.34	0.47	0.54	24.9	26.8
100 mg RP + AM	39.2	32.1	0.60	0.64	0.35	0.37	0.48	0.55	26.4	28.3
50 mg RP + PSB	37.9	28.3	0.53	0.54	0.29	0.32	0.45	0.50	22.7	23.9
100 mg RP + PSB	38.1	31.0	0.55	0.57	0.32	0.34	0.46	0.50	23.7	25.1
50 mg RP + AM + PSB	39.6	33.3	0.61	0.65	0.38	0.41	0.49	0.56	26.7	28.4
100 mg RP + AM + PSB	39.9	35.5	0.63	0.66	0.41	0.44	0.54	0.60	27.8	29.8
new L.S.D. at 0.05 =	4.52	4.93	0.08	0.11	0.15	0.17	0.08	0.10	3.2	3.4

Combined inoculation of AM and PSB at one hundred mg of rock phosphate amendment recorded the highest values as compared to non-inoculated and unamended treatment (control) in both seasons.

These findings are supported by other reports showing that acquisition of mineral nutrients particularly P, N and K, was enhanced in host plants infected with mycorrhizae (Marschner, and Dell, 1994). Also, (Abdel-Hafez, and Abdel-Monsief, 2006) found that VAM colonization is also capable of increasing the nutrient content, which was reflected on higher NPK content of the shoots, roots and fruits of both hosts.

- Shoot content of total carbohydrates

Concerning the effect of treatments on shoot content of total carbohydrates (%), it is apparent from table (3) that the highest increase in the percentage of total carbohydrates (%) of shoots was obtained from the combined inoculation of AM and PSB at one hundred mg of rock phosphate amendment followed by the combined inoculation of AMF and PSB at fifty mg of rock phosphate amendment in comparison with non-inoculated and unamended treatment (control) in both seasons.

The obtained results are in agreement with (Demir, 2004) who showed that pepper plants infected with mycorrhizal fungus Glomus increased sucrose and total sugar content. In addition, (Dixon *et al.*, 1988) on citrus seedlings, found that AM-mycorrhizae increased carbohydrate content of leaves.

In general, the positive effect of AM and PSB inoculations and rock phosphate amendment on chemical characteristics could be explained by that the interaction of phosphate-solubilizing bacteria and arbuscular mycorrhizal fungi can also promote better establishment of arbuscular mycorrhizal fungi by phosphorus ions released from rock phosphate which are taken up by the AM mycelium, thereby maintaining a low soluble phosphorus concentration in the discrete soil microhabitats where the rock phosphate particles are attacked by the phosphate-solubilizing bacteria and thus favoring a continuous phosphorus release (Toro *et al.*, 1997).

Figures (1 & 2 & 3) cleared out the existence of a highly positive correlation between total leaf area per plant (cm^2) and total biomass (g D.W.), between total leaf area per plant (cm^2) and total chlorophyll (mg/g F.W.) and between total leaf area per plant (cm^2) and total carbohydrates (%) of shoot in both seasons.

3. Microbiological studies:

1- AM infection (%):

As concerns with percentage of infection of Thompson Seedless grape rootings with AM-mycrrohizal fungi, data shown in table (4) and illustrated in figure (4) it is obvious that the combined inoculation of AM and PSB at one hundred mg of rock phosphate amendment showed the best infection percentages (85.0 & 100.0%) for both seasons respectively.

This finding indicates that AM-mycrrohizal fungi can solublize the surrounding weatherable mineral through execration of organic acids such as α-Ketoglutraic acid. These organic acids could exert a selective influence on soil microbial communities.

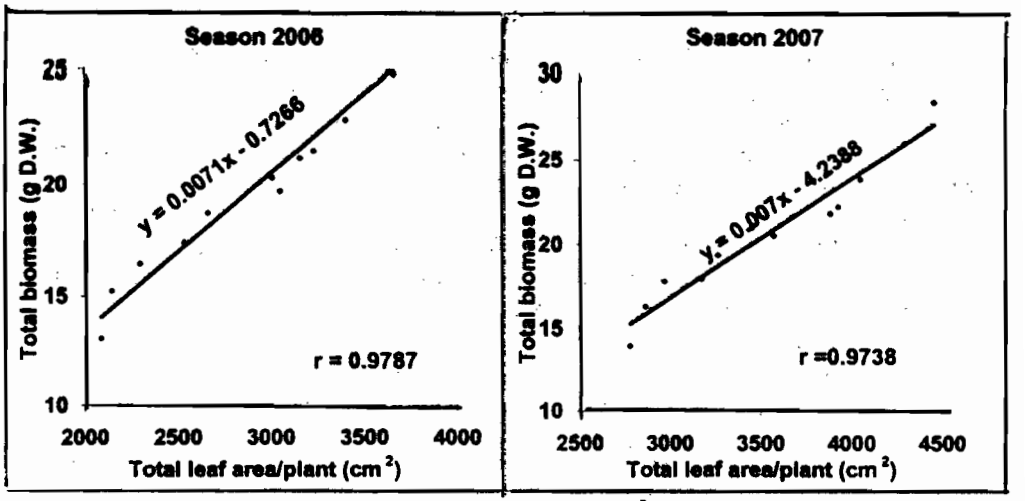

Fig (1): Relationship between total leaf area/plant (cm^2) and total biomass (g D.W.)

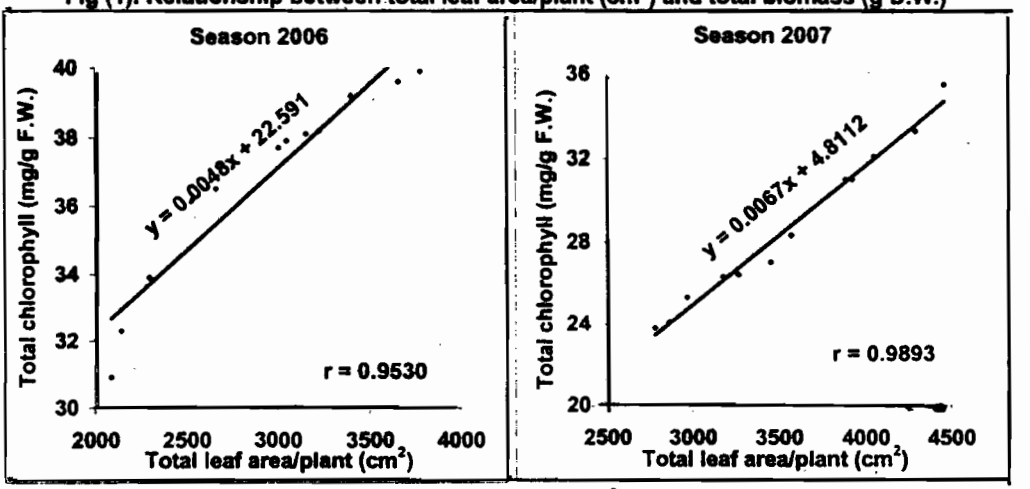

Fig (2): Relationship between total leaf area/plant (cm^2) and total chlorophyll (mg/g F.W.)

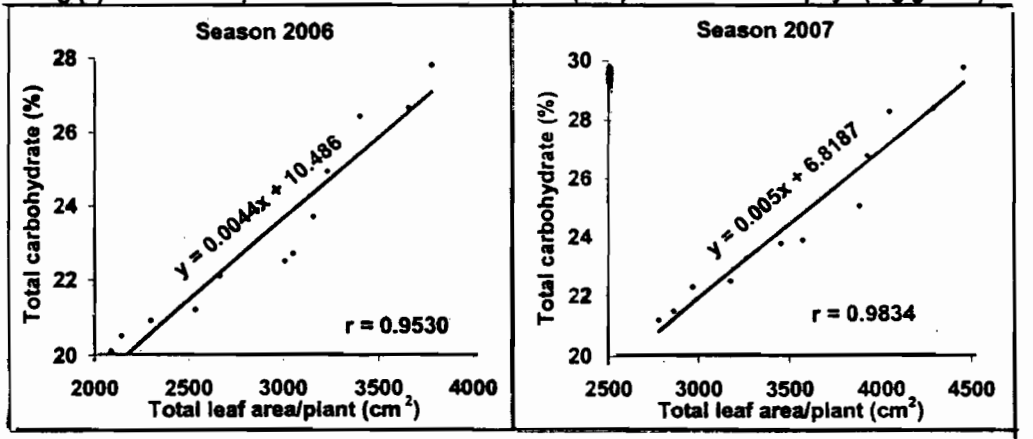

Fig (3): Relationship between total leaf area/plant (cm^2) and total carbohydrates (%)

Table (4): Effect of inoculation with arbuscular mycorrhiza (AM) fungi and phosphate solubilizing bacteria in rock phosphate amended soil on microbiological and enzyme activity in the rhizosphere of Thompson Seedless grape rootings (2006 and 2007 seasons)

Caracteristic / Treatment	AM infection (%)		Number of AM (spore/g soil)		Total count of phosphate solubilizing bacteria (-x10^5 cfu/g soil)		Total microbial count (-x10^{5}cfu/g soil)		Dehydrogenase enzyme activity (µgTPF/g/D.W.soil/day)		Phosphatase enzyme activity (IP/g/D.W.soil/day)	
	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007	2006	2007
Control	20	40	17	30	41.0	41.4	55.0	57.0	29.0	30.1	10.0	10.8
50 mg RP	30	50	25	65	50.1	50.3	70.0	73.6	57.0	57.2	11.1	11.6
100 mg RP	34	52	25	75	63.3	65.0	92.0	98.1	77.0	81.1	13.3	14.2
AM	60	70	310	640	47.3	45.4	64.0	71.0	50.2	52.7	17.4	19.3
PSB	25	45	21	34	51.5	52.0	68.0	70.2	30.7	31.9	15.7	17.9
AM + PSB	65	70	350	760	53.1	54.7	65.0	77.3	55.3	57.4	30.1	33.1
50 mg RP + AM	75	80	430	1000	66.1	66.7	130.0	141.0	84.5	86.6	19.4	22.0
100 mg RP + AM	75	80	560	1070	65.7	70.1	135.0	151.5	96.3	100.1	25.1	27.2
50 mg RP + PSB	30	50	19	42	62.1	63.3	83.0	85.3	33.8	35.8	16.7	18.1
100 mg RP + PSB	40	60	21	55	71.6	84.3	89.0	93.1	60.1	63.3	18.3	22.1
50 mg RP + AM + PSB	80	90	730	1130	81.3	86.6	110.0	115.5	53.7	57.7	31.0	32.0
100 mg RP + AM + PSB	85	100	950	1310	81.7	90.4	166.0	176.0	96.9	103.5	33.5	33.6
new L.S.D. at 0.05 =	17	24	274	291	7.4	9.3	68.3	74.8	47.4	49.3	5.7	6.3

Thereby, phosphate solubilizing bacteria such as Bacillus megaterium proved to be important in the soil inoculated with AM-mycrrohizal fungi since the phosphate-solubilizing rhizobacteria behave as a mycorrhiza helper bacteria (Garbaye, 1994) as they promoted root colonization when associated with mycorrhizal AM-mycrrohizal (Azcon-Aguilar, *et al.*, 1986).

2- Number of AM (spores/g soil):

The results of table (4) and figure (5) show the effect of inoculation with arbuscular mycorrhiza (AM) and PSB at different concentrations (50 or 100mg) of rock phosphate on number of spores/g soil. The obtained results indicated that the combined inoculation of AM and PSB at one hundred mg of rock phosphate amendment gave the highest spore numbers, recording (950 & 1310 spores/g soil) for both seasons respectively, and resulting in an increase over control by (55.9 & 43.7) fold for both seasons respectively.

These findings are In line with those obtained by (Turk *et al.*, 2006) who pointed out that AM-mycorrhizae colonize plant roots and mainly in the surrounding soil extending the roots depletion zone around the root system.

3- Total count of phosphate solubilizing bacteria (-x10^5 cfu/g soil).

Concerning total count of phosphate solubilizing bacteria, data presented in table (4) and illustrated in figure (6) showed that the combined inoculation of AM and PSB at one hundred mg of rock phosphate amendment revealed that the population of PSB reached (81.7 & 90.4 x10^5 cfu/g soil) for both seasons respectively, with an increase over control by (2.0 & 2.2) fold for both seasons respectively.

These results are in the same line with those obtained by (Linderman and Pfleger 1994) who explained that AM-colonization is capable of increasing nutrient content which act as a suitable media for most rhizospheric microorganisms in general and phosphate solubilizing bacteria in particular where AM-mycorrhizae and phosphate solubilizing bacteria contribute to the biochemical cycling of nutrients.

4- Total microbial count (-x10^5cfu/g soil):

Data in table (4) and in figure (7) show the effect of inoculation with arbuscular mycorrhiza (AM) and phosphate solubilizing bacteria at different concentrations (50 or 100mg) of rock phosphate amendment in the soil planted with Thompson Seedless grape rootings on total microbial count. The results indicated that the addition of 100mg of rock phosphate with AM-mycrrohiza and phosphate solubilizing bacteria increased the populations of rhizospheric microorganisms in the roots, recording the highest populations of rhizospheric microorganisms (166.0 & 176.0 x10^5cfu/g soil) for both seasons respectively. The increase over control reached (3.0 & 3.1) fold for both seasons respectively.

The results are in agreement with those obtained by (Godeas *et al.*, 1999) who explained that the increase in populations of rhizospheric microorganism in roots of most plants are influenced by a combined inoculation of microorganism and AM fungi.

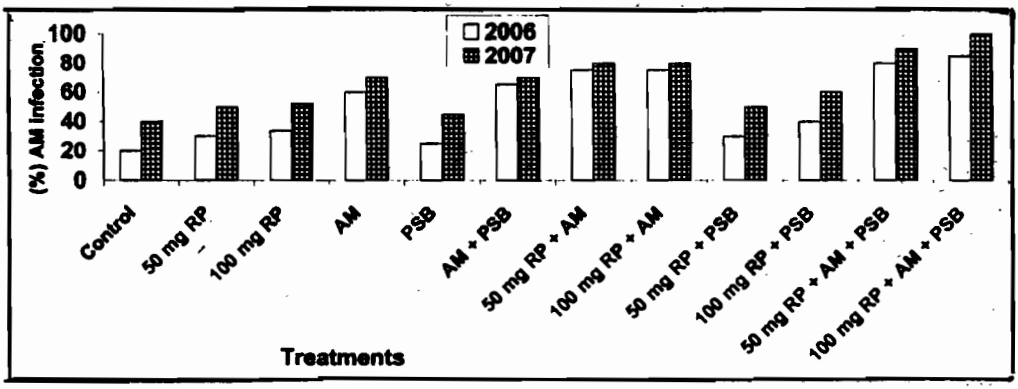

Fig (4): Effect of inoculation with arbuscular mycorrhiza (AM) fungi and phosphate solubilizing bacteria rock phosphate material on AM infection (%) in 2006 and 2007 seasons

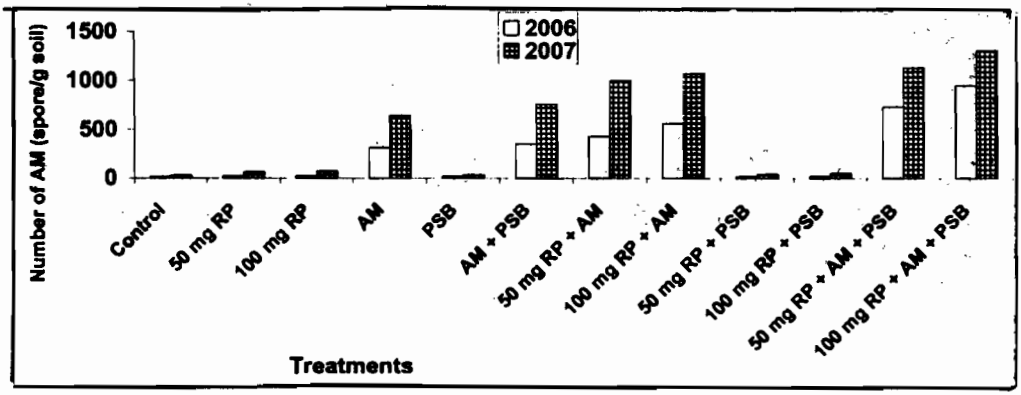

Fig (5): Effect of inoculation with arbuscular mycorrhiza (AM) fungi and phosphate solubilizing bacteria rock phosphate material on number of AM (spore/g soil) in 2006 and 2007 seasons

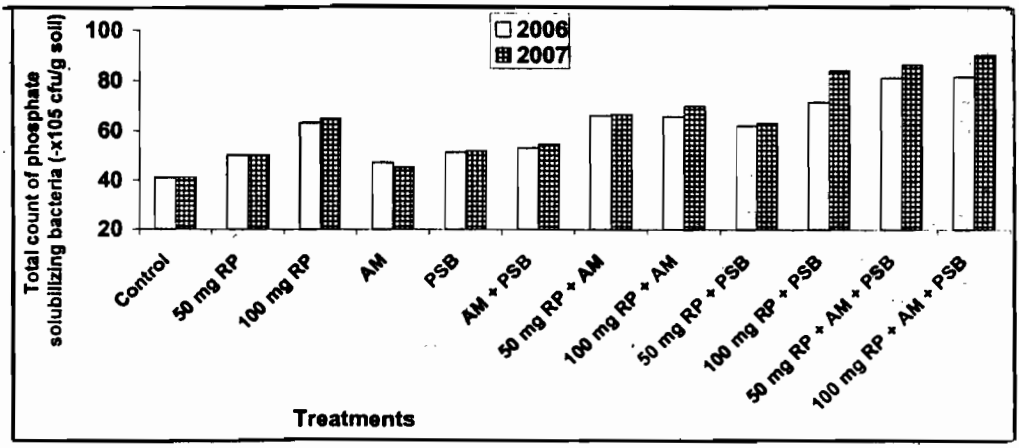

Fig (6): Effect of inoculation with arbuscular mycorrhiza (AM) fungi and phosphate solubilizing bacteria rock phosphate material on total count of phosphate solubilizing bacteria (-x105 cfu/g soil) in 2006 and 2007 seasons

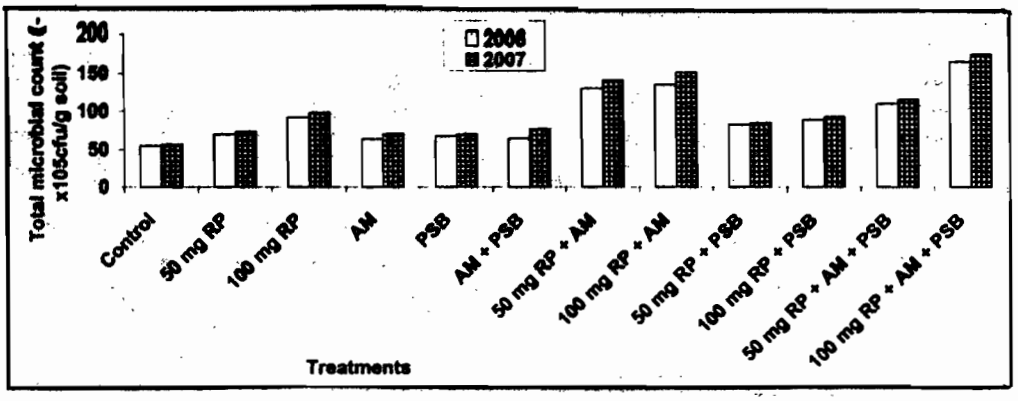

Fig (7): Effect of inoculation with arbuscular mycorrhiza (AM) fungi and phosphate solubilizing bacteria rock phosphate material on total microbial count (-x105cfu/g soil) in 2006 and 2007 seasons

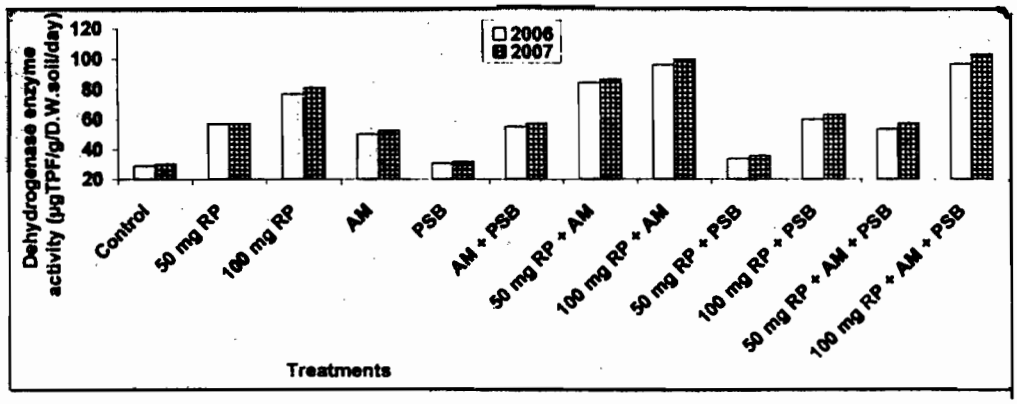

Fig (8): Effect of inoculation with arbuscular mycorrhiza (AM) fungi and phosphate solubilizing bacteria rock phosphate material on dehydrogenase enzyme activity (µgTPF/g/D.W.soil/day) in 2006 and 2007 seasons

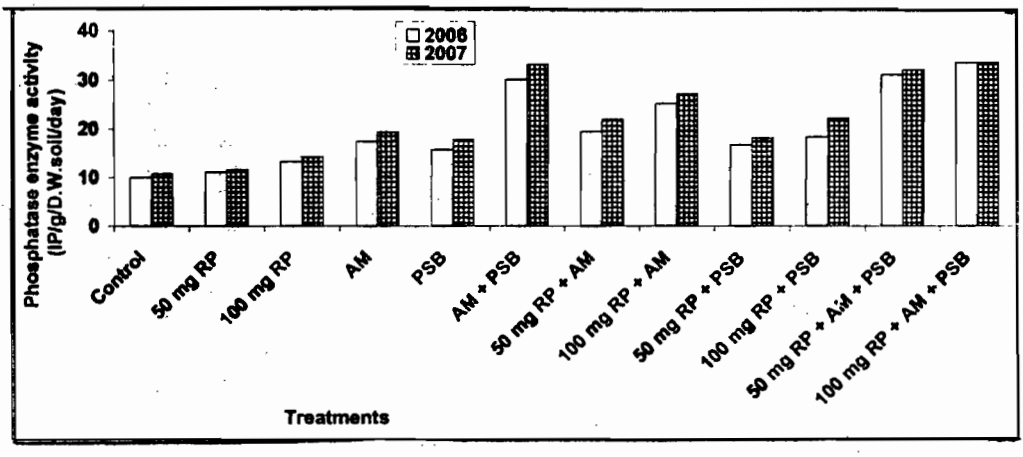

Fig (9): Effect of inoculation with arbuscular mycorrhiza (AM) fungi and phosphate solubilizing bacteria rock phosphate material on phosphatase enzyme activity (IP/g/D.W.soil/day) in 2006 and 2007 seasons

5-Dehydrogenase enzyme activity (µgTPF/g/D.W.soil/day):

Data shown in table (4) and in figure (8) revealed the existence of dehydrogenase enzyme activity among treatments given an indication of microbial activity in the soil inoculated with arbuscular mycorrhiza (AM) and phosphate solubilizing bacteria at different concentrations (50 or 100mg) of rock phosphate amendment. The obtained results indicated that the combined inoculation of AM and PSB at one hundred mg of rock phosphate amendment resulted in the highest activity of dehydrogenase enzyme (96.9 & 103.5 µgTPF/g/D.W.soil/day) for both seasons respectively. The increase in dehydrogenase enzyme activity was attributed to the intense activity of microflora as a mixture of biomass than each individual one. The highest increase in microbial respiration was recorded with the mixture of microorganism in the soil (Massoud, 2005).

6-Phosphatase enzyme activity (IP/g/D.W.soil/day)

As regards phosphatase enzyme activity, data presented in table (4) and illustrated in figure (9) showed that the combined inoculation of AM and PSB at one hundred mg of rock phosphate amendment gave the highest activity of this enzyme (33.5 & 35.5 $x10^5$ IP/g/D.W.soil/day) for both seasons respectively, with an increase over control by (3.2 & 3.3) fold for both seasons respectively.

These results are in agreement with those obtained by (Hetrick, 1989) who found that mycorrhizal hyphae can provide access to insoluble nutrient sources through enzyme activity or some physical or chemical modification of the rhizosphere. The inorganic phosphorus compounds must first be hydrolyzed by phosphatase enzyme which mostly originates from plant roots, through the action of fungi and bacteria.

REFERENCES

Abdel-Hafez, Ahmed A. M. and Abdel-Monsief, Rihan A. (2006): Effects of VA Mycorrhizal inoculation on growth, yield and nutrient content of Cantaloupe and Cucumber under different water regimes. Research Journal of Agriculture and Biological Sciences, 2(6): 503-508.

Asea, P.E.A., Kucey, R.M.N. and Stewart, J.W.B. (1988): Inorganic phosphate solubilization by two Penicillium species in solution culture and soil. Soil Biol. Biochem., 20: 459-464.

Azcon-Aguilar, C., Gianinazzi-Pearson, V., Fardeau, J. C. and Gianinazzi, S. (1986): Effect of vesicular-arbuscular mycorrhizal fungi and phosphate-solubilizing bacteria on growth and nutrition of soybean in a neutral-calcareus soil amended with 32P-45Ca-tricalcium phosphate. Plant Soil 96:3–15.

Barea, J.M., El-Atrach, F. and Azcon, R., (1991): The role of VA mycorrhizas in improving plant N acquisition from soil as assessed with 15N. In: Fitton, C. (Ed.), The Use of Stable Isotopes in Plant Nutrition. Soil Fertility and Environmental Studies, Joint IAEA, FAO Division, Vienna, pp. 677–808.

Bolan, N.S., (1991): A critical review on the role of mycorrhizal fungi in the uptake of phosphorus by plant. Plant and Soil 134, 189–207.

Bouard. J. (1966): Recherches physiologiques sur la vigne et en particulier pour l'aoutment des sarments. Thesis Sc. Nat Bordeaux-France. Pp.34.

Bunt, M.C. and Rovira, A.O. (1955): Microbiological studies of some sub-antarctic soil. J. Soil., 6: 119-128.

Burkert, B., and Robson, A., (1994): ^{65}Zn uptake in subterranean clover (Trifolium subterraneum L.) by three vesicular-arbuscular mycorrhizal fungi in a root free sandy soil. Soil Biol. Biochem. 26, 1117–1124.

Caravaca, F., Alguacil, M.M., Azcon, R., Parlade´, J., Torres, P., and Roldan, A., (2005): Establishment of two ectomycorrhizal shrub species in a semiarid site after in situ amendment with sugar beet, rock phosphate and Aspergillus niger. Microb. Ecol. 49, 73–82.

Chen X., Tang J. J., Zhi G. Y. and Hu S. J. (2005): Arbuscular mycorrhizal colonization and phosphorus acquisition of plants: effects of coexisting plant species. Appl. Soil Ecol. 28:259-269.

Clark R. B. and Zeto S. K. (2000): Mineral acquisition by arbuscular mycorrhizal plants. J. Plant Nutr. 23: 867-902.

Demir, S., (2004): Influence of arbuscular mycorrhiza on some physiological growth parameters of Pepper. Turk J. Biol.28:85-90.

Difco Manual (1984): Dehydrated culture media and reagents for microbiology 10thEd. Difco Laboratories Detroit Michigan, 48232 USA, pp., 621.

Dixon-R.K.; Garrett–HE., and Cox-GS (1988): Carbohydrate relationships of citrus jambhiri inoculated with Glomus fasscicuiatum. Journal of the American Society for Horticultural Science, 113(2): 239 – 242.

Drever, J.L., and Vance, G.F., (1994): Role of soil organic acids in mineral weathering processes. In: Lewan, M.D., Pittman, E.D. (Eds.), The Role of Organic Acids in Geological Processes. Springer-Verlag, New York, pp. 138– 161.

Drobnikova, V. (1961): Factors influencing the determination of phosphatase in soil. Folia Microbial. 6: 260.

Duponnois, R., Colombet, A., Hien, V., and Thioulouse, J., (2005): The mycorrhizal fungus Glomus intraradices and rock phosphate amendment influence plant growth and microbial activity in the rhizosphere of Acacia holosericea. Soil Biol. Biochem. 37, 1460–1468.

Esher, H. and Jensen, V. (1972): Aerobic chemo-organotrophic bacteria of Danish beech forest, OIKOS, 23: 248-260.

Fay P., Mitchell D. T., and Osborne B. A. (1996): Photosynthesis and nutrient use efficiency of barley in response to low arbuscular mycorrhizal colonization and addition of phosphorus. New Phytopathol. 132: 425-433.

Fester T., Maier W. and Strack D. (1999): Accumulation of secondary compounds in barley and wheat roots in response to inoculation with an arbuscular mycorrhizal fungus and co-inoculation with rhizosphere bacteria. Mycorrhiza 8: 241–246.

Gadd, G.M. , (1999): Fungal production of citric and oxalic acid: Importance in metal speciation, physiology and biogeochemical processes. Advances in Microbial Physiology, v.41, p.47-92.

Garbaye, J. (1994): Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol. 128:197–210.

Godeas A.; Fracchia S.; Mujica M.T. and Ocampo J.A. (1999): Influence of soil impoverishment on the interaction between Glomus mosseae and saprobe fungi. Mycorrhiza 9:185-189.

Hetrick. B.A.D., (1989): Acquisition of phosphorus by VA mycorrhizal fungi and the growth responses of their host plants. pp: 205-226. In: L. Boddy, R. Marchant, and D.J. Reid (eds.), Nitrogen, Phosphorus, and Sulphur utilization by fungi. Cambridge University Press, New York, NY, USA.

Jackson, M.L. (1967): Soil Chemical Analysis. Printice-Hall Inc. Englewood Cliffs-N.S.

Knight, W.G., Allen, M.F.; Jurinak, J.J. and Dudley, L.M. (1989): Elevated carbon dioxide and solution P in soil with vesicular-arbuscular mycorrhizal western wheatgrass. Soil Science Society of America Journal 53:1075-1082.

Krishna, H.; Singh, S.K.; Sharma, R.R.; Khawale, R.N.; Grover, M. and Patel, V.B. (2005): Biochemical changes in micropropagated grape (*Vitis vinifera* L.) plantlets due to arbuscular-mycorrhizal fungi (AMF) inoculation during ex vitro acclimatization. Scientia Horticulturae (106): 554—567.

Leyval, C. and Berthelin, J. (1989): Interactions between Lac caria laccata, Agrobacterium radiobacter and beech roots: influence on P, K, Mg and Fe mobilization from mineral and plant growth. Plant and Soil 117:103-110.

Linderman, R. G., and Pfleger, F. L. (1994): General summary. p. 337–344. In F. L. Pfleger and R. G. Linderman (ed.), Mycorrhizae and plant health. APS Press, St. Paul, Minn.

Marschner, H. and Dell, B. (1994): Nutrient uptake in mycorrhizal symbiosis. Plant and Soil. 159:89-102.

Massoud, O.N. (1999): Study on the effect of Azospirillum spp., VA-mycrrohizae and organic matter amended soil on the plant growth. M. Sc. Thesis, department of botany, Faculty of Science, El-Menoufia, University.

Massoud, O.N. (2005): Microbiological and chemical evaluation of compost and its application in organic farming. Ph. D. Thesis, department of botany, Faculty of Science, El-Menoufia, University.

Pregl, F. (1945): Quantitative Organic Micro-Analysis. 4th Ed J. and A. Churchill, Ltd., London.

Raja P. (2006): Status of endomycorrhizal (AMF) biofertilizer in global market. In: Handbook of Microbial Biofertilizers. (Mahendra Rai, ed.), Haworth Press, New York, pp. 383-401.

Reyes, I.; Bernier, L. and Antoun, H. (2002): Rock phosphate solubilization and colonization of maize rhizosphere by wild and genetically modified strains of Penicillium rugulosum. Microbial Ecology, v.44, p.39-48.

Salman, A.C. (1967): Uber die mikrobiello aktivitat und ihr Beziehung Zu Fruchtbartkeits merknmalen. einiger acherboden under besonderer Berucksichtigurg der dehydrogenase akativitat (TTC-Reduktion). Diss Giesen, ph. D. Thesis, W. Germany.

Schreiner, R.P., Mihara, K.L., Mc Daniel, H., and Benthlenfalvay, G.J., (2003): Mycorrhizal fungi influence plant and soil functions and interactions. Plant Soil 188, 199–209.

Singal, R., Gupta, R. and Saxena, Folia R.K. (1994): Rock phosphate solubilization under alkaline conditions by Aspergillus japonicus and A. foetidus. Microbiol., 39: 33-36.

Smith, F., Gilles, M.A., Hamilton, J.K. and Gedess, P.A. (1956): Colorimetric methods for determination of sugar and related substan, Anal. Chem.28, 350.

Snedecor, G. W. and Cochran, W.G. (1980): Statistical Methods. 7th ed., The Iowa State Univ. Press. Ames. , Iowa, U.S.A., pp. 593.

Snell, F.D. and Snell, C.T. (1967): Colorimetric Method of Analysis. D. van Nestrant Company Inc., P. 551-552.

Techapinyawat, S.; Suwannarit, P.; Pakkong, P.; Sinbuathong, N. and Sumthong, P. (2000): Selection of effective vesicle-arbuscular mycorrhiza fungi on growth and nutrient uptake of Vetiver. In:Abstracts of Poster Papers, ICV-2, p 32.

Thompson, J.P., (1987): Decline of vesicular arbuscular mycorrhizas in long fallow disorder of field crops and its expression in phosphorus deficiency in sunflower. Aust. J. Agric. Res. 38: 847-867.

Toro, M., Azcon, R. and Barea, J.M. (1997): Improvement of arbuscular mycorrhiza development by inoculation of soil with phosphate-solubilizing rhizobacteria to improve rock phosphate bioavailability (^{32}P) and nutrient cycling. Appl. Environ. Microbiol, 63: 4408-4412.

Turk M.A.; Assaf T.A.; Hameed R.M. and AL-Tawha A.M. (2006): Significance of myccrohizae world journal of agricultural sciences. 2(1): 16 -20.

Van Der Hejden, M.G.A., Klironomos, J.N., Ursic, M., Moutoglis, P., Streitwolf-Engel, R., Boller, T., Wiemken, A., and Sanders, I.R., (1998): Mycorrhizal fungal diversity determines plant biodiversity ecosystem variability and productivity. Nature 396, 69–72.

Wood, C.W., Reeves, D.W. and Himelrick, D.G. (1992): Relationships between chlorophyll meter readings and leaf chlorophyll concentration. N status and crop yield. A review: Proc. Agro. Soc. N.Z. 23: 1-9.

تلقيح التربة بفطر الميكروهيزا والبكتريا المذيبة للفوسفات كوسيلة لتحسين تيسر صخر الفوسفات لشتلات العنب الطومسون سيدلس

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****قسم بحوث الميكروبيولوجى – معهد بحوث الأراضى والمياه – مركز البحوث الزراعية بالجيزة – مصر**

أجرى هذا البحث بهدف دراسة تأثير التلقيح بكل من فطر الميكروهيزا والبكتريا المذيبة للفوسفات على التربة المضافة إليها تركيزين مختلفين من خام صخر الفوسفات (٥٠ أو ١٠٠ ملجم صخر فوسفات / كجم تربة) بهدف تحسين كلا من النمو الخضرى والحالة الغذائية بالاضافة إلى النشاط الميكروبى والإنزيمى فى منطقة إنتشار الجذور لشتلات العنب الطومسون سيدلس وذلك خلال موسمي ٢٠٠٦ ، ٢٠٠٧.

أعطى التفاعل المشترك بين لقاحى فطر الميكروهيزا والبكتريا المذيبة للفوسفات مع التسميد بمعدل ١٠٠ ملجم صخر فوسفات / كجم تربة أفضل النتائج التى تمثلت فى زيادة جميع الصفات الخضرية تحت الدراسة وهي طول الفرع ، قطر الساق، عدد الأوراق علي الفرع ، مساحة الورقة ، ديناميكية نضج الخشب، الوزن الجاف لكل من المجموع الخضرى والمجموع الجذرى ونسبة المجموع الجذرى إلى المجموع الخضرى، بالإضافة إلى تحسين الحالة الغذائية للشتلات والمتمثلة فى محتوى الأوراق من الكلوروفيل الكلى والنسبة المئوية للنيتروجين والفوسفور والبوتاسيوم ومحتوى الفرع من الكربوهيدرات. أما فيما يتعلق بالنشاط الميكروبى والإنزيمى فى منطقة إنتشار الجذور لشتلات العنب الطومسون سيدلس فقد لوحظ وجود زيادة فى كل من النسبة المئوية للاصابة بالميكروهيزا، عدد جراثيم الفطر، العدد الكلى لخلايا البكتريا المذيبة للفوسفات، العدد الكلى للميكروبات الكلية، نشاط إنزيمات الديهيدروجنيز والفوسفاتيز فى منطقة إنتشار الجذور.