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EFFECT OF MYCORRHIZAL INOCULATION AND PHOSPHATIC FERTILIZATION ON DAMPING-OFF AND ROOT-ROT DISEASES OF SOUR ORANGE.

II- GROWTH CHARACTERS, CHEMICAL ANALYSIS AND COLONIZATION INTENSITY WITH MYCORRHIZAE.

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ABSTRACT: This study was carried out on sour orange seedlings (one-month-old). Seedlings were treated with mycorrhizal fungi (*Glomus macrocarpum*), phosphatic fertilizer and wilting agent disease (*Fusarium oxysporum*) separately or in combination. Data indicated that seedlings inoculated with mycorrhizal fungus alone gave the highest plant height, top and root dry weights, leaf macro elements (N, P, K and Mg) content, and root N and K contents as compared with the other treatments under study. In contrast, *F. oxysporum* caused reducing plant height, dry weight of seedlings, leaf N, P, K and Mg contents as well as root N, P, K and Ca contents than control plants.

On the other hand, mycorrhizal fungus was best than phosphatic fertilizer in improving vegetative growth parameters and leaf & root macro-elements content. Generally, the combined treatment (*G. macrocarpum* + super-P + *F. oxysporum*) gave nearly the same results in dry weight, leaf N and P contents as well as root N, P and K contents comparing with the *G. macrocarpum* + *F. oxysporum* treatment. Also, mycorrhizal fungus with or without phosphatic fertilizer improved vegetative parameters and leaf & root macro-elements content as compared with seedlings treated with *F. oxysporum* fungus alone.

INTRODUCTION

Several workers have observed that tree seedlings with mycorrhizae fungi are more resistant to feeder roots infection by pathogenic fungi than seedlings with few or no mycorrhizae. Marx (1973) showed that plants with mycorrhizae do not exhibit reduce in top growth, chlorosis, restricted root development and eventual death, and are therefore more restricted to feeder root disease than non-mycorrhizal plants. Obviously, an increasing degree of mycorrhizal development induced a proportionate reduction in the amount of feeder roots susceptible to pathogen attack. Also, he suggested that mycorrhizal fungi may produce antifungal, antibacterial and antiviral or metabolites produced symbiotically. Infected host cortical cells may also function as inhibitors to infection and spread of pathogens in mycorrhizal roots. In addition, Zak (1964) postulated several mechanisms by which mycorrhizae may afford disease protection to feeder roots of plants. He suggested that mycorrhizal fungi (a) utilize surplus carbohydrates in the root, thereby reducing the amount of nutrients stimulatory to pathogens, (b) provide a physical barrier (i.e., the fungal mantle) to penetration by the pathogen, (c) secrete antibiotics inhibitory to pathogens, (d) support along with the root, a protective microbial rhizosphere population. Moreover, Stack and Sinclair (1975) reported that the addition of basidiospores of *Laccaria laccota* to nursery soil containing Fusarium oxysporum reduced damping-off (mortality) of Douglas fire seedlings during the first growing season by nearly 100%. The protective influence of *L. laccota* occurred before mycorrhizal formation. Paccua and Milagrosa (1989) found that white potato plants inoculated with *Glomus etunicatum*, *G. macrocarpum* and *Gigospora margarita* fungi increased the yield of potatoes. *Pseudomonas solanacearum* and pathogens infection reduced as the spore concentration of VAM fungi was increased. Likewise, bacterial wilt infection was also decreased. VAM fungi was tested against *Fusarium solani* and showed an increase in yield on mycorrhizal plants and reduced the occurrence of the disease on potatoes.

Moreover, many studies were carried out on the role of mycorrhizal fungi and phosphatic fertilization on growth and minerals content of fruit seedlings. Menge *et al*. (1980), Edrees (1982), Gendiah (1987) and Gendiah *et al*. (1991), reported that mycorrhizal fungi induced an increase in top root growth and some minerals content especially P content. Also, they found that VAM infection rate of plant roots decreased with the increase of soluble P application.

Anyhow, this work was planned in pot experiment to study the effect of mycorrhizal fungi and phosphatic fertilization on wilting disease caused by *Fusarium oxysporum* of sour orange seedlings.

MATERIALS AND METHODS

This research was carried out during 1995 and 1996 seasons in the nursery of Faculty of Agriculture, Moshtohor, Zagazig University.

Early in March, 1995 and 1996, sour orange seedlings (one-month-old) were treated with mycorrhizal fungus (untreated and treated with *Glomus macrocarpum*), phosphatic fertilization (0.0 and 2.0 g P_2O_5/pot) and wilting agent disease (untreated and treated with *Fusarium oxysporum*). Two seedlings were planted in each pot 30 cm diameter filled with a soil mixture of clay and sand (2 : 1 by volume) which was sterilized with 2% formalin solution. All treatments had received 2.0 g K_2O/pot in the form of potassium sulphate. Phosphorus was applied in the form of superphosphate. Potassium and phosphatic fertilizers were mixed with the soil in pots prior to transplanting. Also, each pot under investigation received 6.0 g N/pot in the form of ammonium nitrate in March, May and July.

However, the soil was inoculated with mycorrhizal fungus according to the method of Menge *et al*. (1977). While, *Fusarium oxysporum* fungus (causal of wilting disease) was grown on sterilized sand sorghum grain medium and the fungal inoculum was added to the soil one week before planting of seedlings according to Whithead (1975) method.

Generally, treatments used in this study were arranged in randomized complete block design. Each treatment was replicated 8 times (2 seedlings/pot).

Plant height (cm), top and root dry weight (g) were estimated at the end of experiment in October. The dried material of both leaves and roots were used for determining N, P and K nutrients as percentages according to Pregl (1945), Matt (1968) and Brown and Lilliland (1946) methods, respectively. While, Ca and Mg contents were estimated according to Chapman and Pratt (1961) method.

Also, infection and intensity tests with mycorrhizal fungus were conducted according to the procedure of Hayman (1970).

All obtained data were transformed into angles to be statistically analyzed (Steel and Torrie, 1960). The Duncan's multiple range test (Duncan, 1955) was used to differentiate among means.

RESULTS AND DISCUSSION

I- Mycorrhizal fungi, phosphatic fertilization and causal wilting disease as related to :

1- Plant height and dry weight of sour orange seedlings :

Data presented in **Table (1)** clearly show that mycorrhizal fungus (*G. macrocarpum*) caused a significant increase in plant height, top dry weight, root dry weight and whole plant dry weight than non-mycorrhizal plants (control). The same effect was obtained by Gerdmann (1975), Edrees (1982), and Gendiah *et al.* (1991).

Moreover, phosphatic fertilization increased plant height and top & root dry weight of sour orange seedlings as compared with control seedlings.

On the other hand, *Fusarium oxysporum* caused reducing in plant height, top dry weight and whole plant dry weight than the analogous one of control seedlings. This result is clearly shown in **Fig. (1).** Similar results were found by Marx (1973) and Stack & Sinclair (1975).

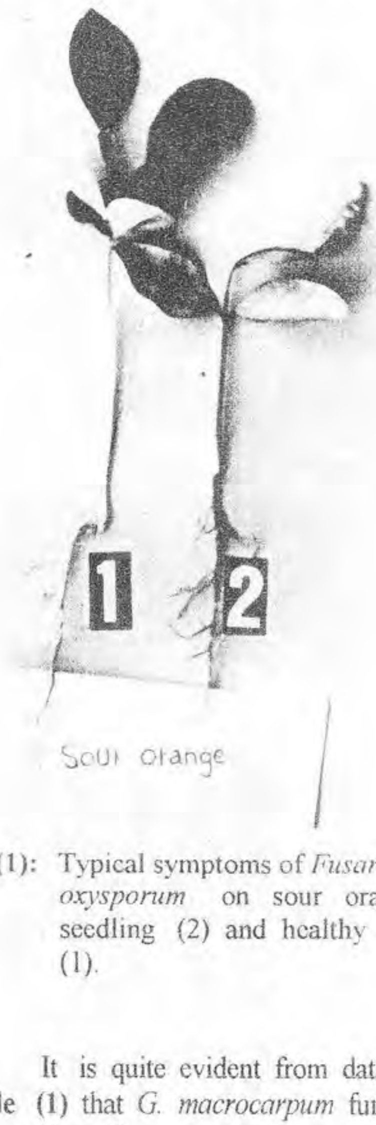

Fig.(1): Typical symptoms of *Fusarium oxysporum* on sour orange seedling (2) and healthy one (1).

It is quite evident from data in **Table (1)** that *G. macrocarpum* fungus with either *F. oxysporum* or super-P increased plant height, top dry weight, root dry weight and whole dry weight of seedlings as compared with control or seedlings treated with *F. oxysporum* alone. On the other hand, phosphatic fertilization with *F. oxysporum* gave similar results in the plant height and dry weight of plants as compared with control or *F. oxysporum* treatment alone. Also, the interaction

Table (1): Effect of the interaction between mycorrhizae, phosphatic fertilizer and causal wilting disease on plant height and dry weight of sour orange seedlings.

Treatment	Plant height (cm)		Top dry weight (g/plant)		Root dry weight (g/plant)		Whole plant dry weight (g)	
	1995	1996	1995	1996	1995	1996	1995	1996
Control	23.3 c	18.3 d	2.12 d	2.17 b	1.90 bc	1.31 a	4.02 de	3.48 cd
G. macrocarpum	29.7 a	27.0 a	4.22 a	3.59 a	2.93 a	1.73 a	7.15 a	5.32 a
Super-P	25.7 b	19.7 d	2.39 cd	2.25 b	2.42 ab	1.37 a	4.81 cd	3.62 bc
Fusarium oxysporum	19.7 d	16.0 e	1.81 d	1.77 c	1.32 c	0.71 b	3.13 e	2.48 e
G. macrocarpum + Super-P	27.3 b	25.7 ab	3.70 ab	2.40 b	2.53 ab	1.70 a	6.23 ab	4.10 b
G. macrocarpum + *F.oxy.*	20.0 b	22.3 c	3.15 bc	2.32 b	2.58 ab	1.62 a	5.73 bc	3.94 bc
F. oxysporum + Super-P	22.3 c	19.7 d	2.10 d	1.68 c	2.12 abc	1.35 a	4.22 de	3.03 de
G. mac. + Super-P + *F. oxy.*	23.0 c	24.3 bc	2.67 bcd	2.18 b	2.53 ab	1.65 a	5.20 bc	3.83 bc

Means followed by the same letter(s), within each column, are not significantly different from each other at 1% level.

between *G. macrocarpum*, super-P and *F. oxysporum* was more effective in increasing plant height and whole plant dry weight than seedling treated with *F. oxysporum* alone. Generally, the lowest values of plant height and dry weights of seedlings were obtained in seedlings treated with *F. oxysporum* alone. These results are in agreement with the findings of Zak (1964) and Paccua & Milagrosa (1989).

2- Leaf macro-elements content of sour orange seedlings:

Data in **Table (2)** expressed that seedlings inoculated with *G. macrocarpum* fungus gave the highest N, P, K and Mg content of sour orange leaves than non-mycorrhizal seedlings. The contrast was true when leaf Ca content was concerned. These results are in agreement with Menge *et al.* (1980), Edrees (1982), Gendiah (1987) and Gendiah *et al.* (1991).

Moreover, super-P application to sour orange seedlings induced a significant increase in leaf P and Mg contents comparing with unfertilized seedlings.

On the other hand, *F. oxysporum* fungus caused a significant decrease in leaf N and Mg contents of seedlings infested with it alone as compared with uninfested plants (control). Also, *F. oxysporum* treatment reduced leaf P and K contents comparing with control seedlings, while the differences of leaf P and K values didn't reach a significant level between them. In contrast, *F. oxysporum* fungus is more effective in increasing leaf Ca content than those of seedlings uninfested with the same fungus (control). Similar results were obtained by Stack & Sinclair (1975) and Paccua & Milagrosa (1989).

The data in **Table (2)** also cleared that mycorrhizal fungus plants either fertilized with P or treated with *F. oxysporum* gave higher N, P, K and Mg contents of sour orange leaves as compared with the analogous ones of seedlings treated with *F. oxysporum* alone.

The interaction between *F. oxysporum* and phosphatic fertilizer was more effective in increasing leaf K and Mg contents than *F. oxysporum* alone treatment, while leaf N and P contents didn't show significant level in differences between them. Moreover, *G. macrocarpum* + *F. oxysporum* + super-P treatment increased leaf N, P, K and Mg contents as compared with seedlings treated or untreated with *F. oxysporum*. Generally, *F. oxysporum* fungus increased leaf Ca content than other treatments under study. Those results are in agreement with findings of Marx (1973) and Stack & Sinclair (1975).

3- Root macro-elements content:

Data in **Table (3)** clearly show that mycorrhizal fungus treatment gave the same significant letters in root N, P, K, Ca and Mg contents in both seasons of study as compared with the control plants except root K (in the second season) and Ca (in the first season) contents increased than control. Similar results were obtained by Gendiah (1987) & Gendiah *et al.* (1991).

Super-P application to soil reduced root N, P and K contents than control seedlings treatment. While, root Mg content increased in super-P treatment than control, but it had no effect on Ca content. On the other hand, *F. oxysporum* fungus treatment reduced root N, P, K and Ca contents as compared with control treatment. Sour orange seedlings infested with *F. oxysporum* fungus gave the

Table (2): Effect of the interaction between mycorrhizae, phosphatic fertilizer and causal wilting disease on leaf macro-elements content of sour orange seedlings.

Treatment	N%		P%		K%		Ca%		Mg%	
	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
Control	2.00 ab	2.10 ab	0.20 cd	0.18 c	1.26 c	1.35 cd	4.0 b	3.8 b	1.32 b	1.28 b
G. macrocarpum	2.20 a	2.40 a	0.30 a	0.32 a	1.96 a	2.15 a	3.6 b	3.2 c	2.04 a	2.16 a
Super-P	1.80 bc	2.00 bc	0.30 a	0.28 ab	1.34 bc	1.40 c	4.0 b	3.9 b	1.92 a	1.98 a
Fusarium oxysporum	1.60 c	1.70 c	0.15 d	0.16 c	1.00 c	1.08 d	5.4 a	4.9 a	0.96 c	0.86 c
G. macrocarpum + Super-P	2.00 ab	2.00 bc	0.22 bcd	0.23 bc	1.20 c	1.40 c	5.2 a	4.8 a	1.92 a	1.95 a
G. macrocarpum + *F.oxy.*	2.00 ab	2.10 ab	0.26 abc	0.28 ab	1.28 c	1.38 cd	3.0 c	3.2 c	1.28 b	1.30 b
F. oxysporum + Super-P	1.80 bc	1.70 c	0.18 cd	0.17 c	1.70 ab	1.80 b	3.8 b	3.6 b	1.44 b	1.52 b
G. mac. + Super-P + *F. oxy.*	2.20 a	2.30 ab	0.29 ab	0.28 ab	2.00 a	2.05 ab	3.8 b	3.8 b	2.16 a	2.12 a

Means followed by the same letter(s), within each column, are not significantly different from each other at 1% level.

Table (3): Effect of the interaction between mycorrhizae, phosphatic fertilizer and causal wilting disease on root macro-elements content of sour orange seedlings.

Treatment	N%		P%		K%		Ca%		Mg%	
	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
Control	2.2 a	2.10 a	0.23 ab	0.21 ab	1.7 a	1.5 b	2.0 c	2.2 cd	0.72 d	0.84 de
G. macrocarpum	2.0 ab	1.95 a	0.21 b	0.18 bc	1.8 a	1.7 a	2.4 b	2.3 cd	0.72 d	0.68 e
Super-P	1.6 c	1.60 b	0.18 b	0.15 c	1.1 b	1.2 cd	2.0 c	2.1 d	0.96 c	1.02 cd
Fusarium oxysporum	1.6 c	1.70 b	0.20 b	0.17 bc	0.9 b	1.0 d	2.4 b	2.4 bcd	1.68 a	1.60 a
G. macrocarpum + Super-P	1.6 c	1.60 b	0.21 b	0.18 bc	1.9 a	1.9 a	2.6 b	2.5 bc	1.16 bc	1.22 bc
G. macrocarpum + *F.oxy.*	1.6 c	1.68 b	0.18 b	0.17 bc	1.1 b	1.3 bc	3.4 a	3.1 a	1.18 bc	1.20 bc
F. oxysporum + Super-P	1.8 bc	2.0 a	0.28 a	0.26 a	1.1 b	1.3 bc	2.4 b	2.6 b	1.32 b	1.40 ab
G. mac. + Super-P + *F. oxy.*	1.8 bc	2.0 a	0.18 b	0.17 bc	0.9 b	1.2 cd	2.0 c	2.2 cd	0.72 d	0.92 d

Means followed by the same letter(s), within each column, are not significantly different from each other at 1% level.

highest root Mg content as compared with the other treatments under study.

The addition of calcium superphosphate with mycorrhizal fungus caused a reduction in root N and Mg contents, and had no effect on root P content as compared with control seedlings. On the other hand, *G. macrocarpum* + super-P treatment gave the highest values of root K content. While, the same treatment induced an increase in root Ca content than control. Gendiah (1987) found similar results.

Concerning the effect of the interaction between *G. macrocarpum* and *F. oxysporum* fungi on root macro-elements content, it increased root K content as compared with control plants. Also, it gave the highest values of root Ca content than the other treatments under study. While, *G. macrocarpum* + *F. oxysporum* treatment had no effect on root N and P contents as compared with *F. oxysporum* treatment. In contrast, it reduced root Mg content than seedlings infested with *F. oxysporum* fungus. Zak (1964) and Paccua & Milagrosa (1989) had found this trend.

Seedlings treated with *F. oxysporum* + phosphatic fertilizer gave the highest values of root P content than the other treatments. Also, similar trend was found in root N and K of seedlings infested to *F. oxysporum* and the addition of phosphatic fertilizer. While, combined treatment had no effect on root Ca content as compared with *F. oxysporum* treatment. On the other hand, root Mg content reduced in seedlings treated with combined treatment as compared to *F. oxysporum* treatment. Husien (1996) obtained similar results.

The combined treatment (*G. macrocarpum* + *F. oxysporum* + super-P) improved root N content as compared to seedlings infested with *F. oxysporum* fungus alone. Also, combined treatment gave similar results in root N, P, Ca and Mg contents, while root K content reduced as compared withcontrol plants. Husien (1996) found approximately similar results.

II- Infection percentage and intensity of endomycorrhizal fungus in sour orange roots as influenced by phosphatic fertilizer and causal wilting disease.

The data in **Table (4)** indicated that the infection percentage of mycelium, vesciles and arbscules of mycorrhizal fungus reached the maximum (100%) in roots of sour orange seedlings inoculated with *G. macrocarpum* in October. The same results were found by Gendiah (1987) & Gendiah *et al.* (1991).

Moreover, number of vesciles and arbscules of *G. macrocarpum* fungus decreased in roots by phosphatic fertilization. Also, *F. oxysporum* fungus was more effective in reducing mycelium, vesciles and arbscules numbers in root than phosphatic fertilizer. Generally, *G. macrocarpum* + *F. oxysporum* + super-P treatment gave the second rank in numbers of mycelium, vesciles and arbscules in sour orange roots. The highest numbers of mycelium, vesciles and arbscules were found in seedling roots which were treated with *G. macrocarpum* fungus alone. Similar results were obtained by Husien (1996).

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Table (4): Infection percentage and intensity of endomycorrhizal fungus in sour orange roots as influenced by phosphatic fertilizer and causal wilting disease.

Treatment	Infection (%)						Intensity					
	Mycelium		Vesecals		Arbuscles		Mycelium		Vesecals		Arbuscles	
	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
Control	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c	0.0 d	0.0 d	0.0 d	0.0 c	0.0 d
G. macrocarpum	100 a	100 a	100 a	100 a	100 a	100 a	3.2 a	3.1 a	28.1 a	23.9 a	8.3 ab	13.3 a
Super-P	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c	0.0 d	0.0 d	0.0 d	0.0 c	0.0 d
Fusarium oxysporum	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c	0.0 d	0.0 d	0.0 d	0.0 c	0.0 d
G. macrocarpum + Super-P	100 a	100 a	100 a	100 a	100 a	100 a	2.8 a	2.9 a	17.6 b	17.4 b	9.7 a	9.2 b
G. macrocarpum + *F.oxy.*	100 a	100 a	100 a	100 a	100 a	100 a	1.7 b	1.2 c	12.9 c	13.3 c	7.5 b	4.5 c
F. oxysporum + Super-P	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 b	0.0 c	0.0 d	0.0 d	0.0 d	0.0 c	0.0 d
G. mac. + Super-P + *F. oxy.*	100 a	100 a	100 a	100 a	100 a	100 a	2.0 b	2.3 b	16.7 b	18.6 b	8.7 ab	9.2 b

Means followed by the same letter(s), within each column, are not significantly different from each other at 1% level.

تأثير التلقيح بفطريات الميكورهيزا والتسميد الفوسفاتي على أمراض موت البادرات وعفن الجذور في النارنج

٢ – صفات النمو والتحليل الكيميائي وكثافة فطر الميكورهيزا في جذور النارنج

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تمت هذه الدراسة على شتلات النارنج (عمر شهر واحد) والتي عوملت بفطر الميكورهيزا (*Glomus macrocarpum*) والتسميد الفوسفاتي ومسبب مرض الذبول (*Fusarium oxysporum*). أشارت النتائج إلى أن الشتلات الملقحة بفطر الميكورهيزا بمفرده أعطت أعلى أطوال للنباتات والوزن الجاف للمجموع الخضري والجذري ومحتوى الأوراق من النيتروجين والفوسفور والبوتاسيوم والمغنسيوم وكذا محتوى الجذور من النيتروجين والبوتاسيوم وذلك مقارنة بجميع المعاملات الأخرى تحت الدراسة.

على العكس وجد أن فطر الفيوزاريوم كان أكثر تأثيرا في تقليل أطوال النباتات والوزن الجاف للشتلات ومحتوى الأوراق من النيتروجين والفوسفور والبوتاسيوم والمغنسيوم ، وكذا محتوى الجذور من النيتروجين والفوسفور والبوتاسيوم والكالسيوم مقارنة بنباتات الكنترول.

على الجانب الآخر كان فطر الميكورهيزا أفضل من التسميد الفوسفاتي في تحسين النمو الخضري ومحتوى الأوراق والجذور من العناصر الكبرى. عموما المعاملة الخليطة (*Glomus macrocarpum* + سوبرفوسفات + *Fusarium oxysporum*) أعطت تقريبا نفس النتائج في الوزن الجاف ومحتوى الأوراق من النيتروجين والفوسفور وكذا محتوى الجذور من النيتروجين والفوسفور والبوتاسيوم مقارنة بمعاملة فطر *Glomus macrocarpum*+ فطر *Fusarium oxysporum*. أيضا فطر الميكورهيزا سواء مع أو بدون التسميد الفوسفاتي حسن من قياسات النمو الخضري ومحتوى الأوراق والجذور من العناصر الكبرى مقارنة بالشتلات المعاملة بفطر *Fusarium oxysporum*.