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EFFECT OF BIOFERTILIZATION AND BIOLOGICAL CONTROL ON GROWTH AND CHEMICAL CONSTITUENTS OF VOLKAMERIANA SEEDLINGS.

BY

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ABSTRACT

Recently, Volkameriana seedlings rootstock are introduced to Egypt for propagation of citrus trees.

In this research endomycorrhizae (*Glomus macrocarpum*), biological control agent (*Trichoderma harzianum*), *Rhizoctonia solani* fungi and combination of them were used to study the effect of inoculation with *G. macrocarpum* and *T. harzianum* in the presence of *R. solani* on growth and chemical constituents of volkameriana

Obtained results showed that total microbial flora and actinomycetes populations were increased with the increasing of growth period of volkameriana to reach their maximum values during the period ranged between 60-90 days and 120-150 days for total microbial flora and actinomycetes counts, respectively then gradually decreased thereafter. In contrast, the counts of total fungi in rhizosphere region gradually decreased with the increasing of growth period in various investigated treatments.

The highest populations of total microbial flora were observed in the treatment of *G. macrocarpum* combined with *R. solani* + *T. harzianum*. Highest actinomycetes populations were observed in the treatment included mycorrhizal fungus alone. Whereas, the highest count of fungi was observed in the treatments included either *R.solani* alone or untreated one (control). Inoculation with either *G.macrocarpum* or *T. harzianum* as well as the combination of them led to sharp decrease in the populations of fungi.

Volkameriana seedlings inoculated with mycorrhizae and *T. harzianum* either in infested or uninfested soil with *R. solani* showed a significant increase

in plant height and top and root dry weights as well as in leaves contents of chlorophyll a and b during two seasons of study.

Statistical analysis clearly showed that mycorrhizal inoculation increased N, P, K, Mg and Ca contents of leaves and roots as compared to control (uninoculated plants). However, *T. harzianum* fungus had no effect on leaves and roots macro -nutrients content in comparison with the control plants. In contrast, *R. solani* alone reduced the values of macro-nutrients content of leaves and roots. In general, combination treatment (*T. harzianum* + *G. macrocarpum*+ *R. solani*) improved leaves and roots macro - nutrients content as compared to either *T. harzianum* + *R. Solani* or *G. macrocarpum* + *R. solani* treatments.

The infection percentage and colonization intensity of mycorrhizae fungus reached to the highest level in root seedlings inoculated with *G. macrocarpum* + *T. harzianum* + *R. solani*. Obtained results clearly show that mycorrhizal inoculation either alone or in combination with T. *harzianum* in the presence of *R. Solani* improved growth characters and chemical constituents of Volkameriana plants. This result could be attributed to *G. macrocarpum* and *T. harzianum* can antagonize *R. Solani* and consequently reduce its harmful effect.

INTRODUCTION

Citrus rootstock seedlings are well known to be attack with various fungi especially damping-off and root-rot fungi in the nursery (Fig., 1). Volkameriana lime seedlings is considered a new rootstok in Egypt able to resist many diseases especially virus diseases and also the scion grew on it vigorously and healthy. In addition, it is suitable for many soils.

In this respect, two ways could be used in biological control for plants : The first way, application of microbial metabolic substances which increase plant resistance against parasitic fungi. This treatment is effective in particular under field conditions. While, the second way is mycorrhizal fungi inoculation (as a biofertilizer) or *Trichoderma spp.* (as a biological control) since the interactions between mycorrhizal fungi, *Trichoderma* and other soil microorganisms caused damping - off and root- rot diseases for many plants widely occurred. Wingfield (1968) observed that ectomycorrhizae formed by *Pisolithus tinctorius* fungus on axenic seedlings of *P.taeda* enhanced their survival when grew with the root- rot pathogen *Rhizoctonia solani*. Baltruschat and Schonbeck (1972) observed fewer chlamydospores of *Thielaviopsis basicola* formed on mycorrhizal than on non- mycorrhizal roots of tobacco inoculated with the pathogen. The number of spores were inversely correlated with the amount of VAM infection. Mycorrhizal root extracts added to malt agar cultures of *T. basicola* inhibited chlamydospores production by 80 to 100%. This was largely attributed to the amino acid arginine which accumulated in the endomycorrhizae. Baltruschat *et al.*, (1973) found that the pathogens decreased

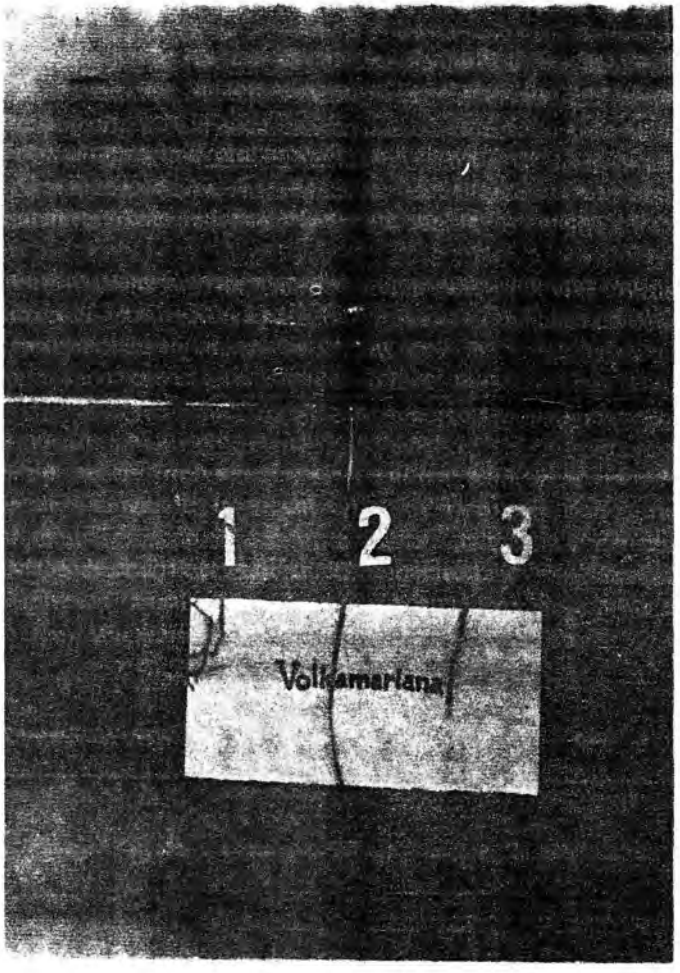

Fig. (1): Volkameriana seedlings.

1-Healthy seedling **2-Diseased seedling**

the weights of non- mycorrhizal plants by 64% while weights of mycorrhizal plants by only 28%.

On the other hand, a few workers studied the interaction between biological control fungi and mycorrhizal fungi. Koehl and Schloesser (1989) pointed out that *Trichoderma spp.* have a high antagonistic potential against numerous pathogenic fungi in pot experiments. However, an infection and colonization of maize roots by autochthonous VAM fungi of a field soil or additional inoculm of *Glomus etunicatum* were almost unaffected by both *T. hamatum* and *T. harzianum*. Thus, a combined application of VAM and *Trichoderma spp.* to promote plant growth appears to be feasible. Regarding the effect of biofertilization on microbial counts, Azazy *et al.*, (1988) revealed that biofertlization with phosphate dissolvers increased the total bacterial count, actinomycetes and asymbiotic N_2-fixers.

Mycorrhizal fungi increased the growth, nutrients content and growth promoting substances production in the host plants (Menge, *et al.*, 1980; Edrees, 1982 and Gendiah, *et al.*, 1991. Also, *Bellone* and *deBellone*, 1993) found that mycorrhizal fungi increase the availability of most nutrients specially P and some micro-elements which encouraged the proliferation of different soil micro- organisms.

Therefore, the purpose of this research is to study the effect of biofertilization (mycorrhizae) and biological control (*Trichoderma harzianum*) on growth of volkameriana seedlings infested with damping off and root-rot pathogen (*Rhizoctonia solani*). As well as study the effect of these treatments on some microbial activities and VAM colonization intensity.

MATERIALS AND METHODS

A pot experiment was carried out to evaluate the effect of biofertilization and biological control on growth and some chemical constituents of volkameriana rootstock (*Citrus volkameriana L.*).

This research was carried out during 1995 and 1996 seasons at the Fac. of Agric. Moshtohor, Zagazig Univ.,. In early March, seedlings were planted in pots of 30 cm in diameter, filled with loamy clay soil then the pots were treated as follows:

1. Control.
2. *Glomus macrocarpum* fungus (*Mycorrhizae*).
3. *Trichoderma harzianum* fungus (biological agent).
4. *Rhizoctonia solani* fungus (causal damping-off disease).
5. *G. macrocarpum* + *T. harzianum*.
6. *G. macrocarpum* + *R. solani*.
7. *T. harzianum* + *R. solani*.
8. *G. macrocarpum* + *T. harzianum* + *R. solani*.

Rhizoctonia solani and *Trichoderma harzianum* fungi were kindly supplied from Plant Pathology Institute, Agric. Res. Center, Giza, Egypt. In addition, inoculum of *R. solani* was prepared by using the sand sorghum grain medium (Withead, 1975). While, inoculum of *T. harzianum* was prepared on liquid gliotoxin fermentation medium (Brain and Hemming, 1945).

The soil was inoculated with vesicular arbuscular mycorrhizae (VAM) and biological control agent fungi at planting time while, the soil was infested by causal damping-off fungus one week before seedlings plantation. The soil was irrigated to activate the growth of pathogenic fungus *R. solani*.

Mycorrhizal fungus (*Glomus macrocarpum*) was added according to Menge *et al.*, (1977) method. While, *Trichoderma harzianum* fungus inoculum was added according to Abd El-Moity laccording to (1986). Also, *Rhizoctonia solani* fungus was added at a rate 3% of soil weight according to Eisa, *et al.*, (1994)..

Generally, each pot received annually 6.0 gm N in the form of Ammonium nitrate in March, May and July, Phosphorus and Potassium fertilizers were added at the rates of 1.0 gm P_2O_5 and 1.5 K_2O per pot in the form of Calcium superphosphate and Potassium sulphate at sowing time as soil application. Anyhow, treatments were arranged in a randomized complete block design and each treatment was replicated 8 times with two seedlings per pot.

Determinations:

A- Microbiological analyses:

Rhizosphere soil samples of the developed plantlets were taken monthly and microbiologically analyzed for total microbial flora, actinomycetes and fungi counts.

Soil yeast extract agar medium was used for counting of total microbial flora (Skinner *et al.*, 1952). While, Jensen medium was used for actinomycets count and prepared as described by Allen (1952). Besides, Martin's medium (1950) was used for counting the fungi.

Anyhow, the plates count method was used in the three determinations.

II- Growth parameters and chemical analyses:

In late October, in each season, seedlings of volkameriana were carefully removed from pots and subjected to the following measurements: plant height (cm), top and root dry weights (gm), leaf chlorophyll a and b contents, leaf and root macro-elements content (N, P, K, Ca, and Mg) as a percentage. Nitrogen, Phosphorus and Potassium contents were determined according to Kjeldahl digestion method as described by Jackson (1973), Matt (1968) and Brown & Lilliland (1946) methods, respectively. While, Calcium and Magnesium contents were estimated according to Chapman and Pratt (1961)

method. Leaf chlorophyll content was determined according to Van Wettstein (1957) method.

C- Infection percentage and colonization intensity of VAM fungus:

Infection and colonization intensity of VAM fungus were carried out on seedlings roots at the termination of the experiment according to Hayman and Mosse (1970) method.

Lastly, the Duncan's multiple range test (Duncan, 1955) was used to differentiate among means. Moreover, all percentages were transformed into angles to be statistically analyzed according to Steel and Torrie (1960) method.

RESULTS AND DISCUSSION

A- Microbiological analyses:

A-1. Periodical changes in total microbial flora counts in the rhizosphere of volkameriana rootstock:-

Data presented in Table (1) show that changes in total microbial flora in the rhizosphere of volkameriana rootstock at different determination periods as well as under different treatments. Counts of total microbial flora show fluctuations during growth period. These fluctuations were most probably due to the temperature changes occurring in the greenhouse. Total microbial flora gradually increased with the increasing of growth period to reach their maximum values at 60 - 90 days then decreased thereafter. These results were true in all treatments and both growing seasons. Counts of total microbial flora were higher in the treatments included mycorrhizal inoculation than ones which uninoculated with VAM fungus (*G. macrocarpum*) and this was obvious in case of *G. macrocarpum* combined with *R. solani* + *Trichoderma harzianum* treatment.

This result could be attributed to the mycorrhizal fungi produce growth promoting substances as well as increased the availability of most nutrient elements especially Phosphorus and micro-nutrients which enhanced the bacterial proliferation in soil Azazy *et al.*, (1988) and Bellone and de Bellone (1993). Discarding the control, the lowest counts of total microbial flora resulted from soil infested with *R. solani* only. This result may be due to the antagonistic effect of *R. solani* on different soil bacteria. Also, comparing the control, obtained data reveal that *T. harzianum* addition led to an increase in total microbial flora in the rhizosphere of volkameriana rootstock. Generally, total microbial flora counts were higher in the 2nd season than in the 1st one and this may be due to the difference in meterological factors (climatic conditions) between the two seasons.

Table (1): Periodical changes in total microbial counts (x 10^6 /g dry weight of soil) in the rhizosphere of volkameriana rootstock.

Period (days) / Treatments	Initial		30		60		90		120		150		180	
	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
Control	32.5	50	72	80	98	102	30	42.2	22.3	24	36.5	48	32.8	38.7
G. macrocarpum	140.2	160.1	180	200	320.8	300	260	280	180.8	200	120.5	160	150.5	120.3
T. harzianum	64.8	72	140	160	150	180	100	120	60.6	80	41.7	48.3	44.5	56.6
Rhizoctonia solani	48.3	52	78	82	90	97	44.3	48	28.5	40	30.3	38	34.3	41.6
G. Macrocarpum + T. harzianum	84	92	120.2	140.3	161.8	165	178	184	168	190	156.6	170	90.7	95.8
G. macrocarpum + R. solani	92	98	160.2	180	120	156	180	186	108	120	142.4	152	84.6	88.7
T. Harzianum + R. solani	56	60	90.7	110	140	160.2	186	192	132	140	148.3	162	64.3	72.2
G. Macrocarpum + T. harzianum + R. solani	158	164	260	280	320	360	280	300	240	340	183.8	200.7	112.2	120.6

A-2. Periodical changes in actinomycetes counts in the rhizosphere of volkameriana rootstosk:

Data shown in Table (2) clearly indicate that the actinomycetes counts were differed by different treatments and growth periods. Actinomycetes counts showed fluctuations during growth period under various treatments. Populations of actinomycetes gradually increased with the increasing of growth periods to reach their maximum values at the period ranged between 120 -150 days. The highest population of actinomycetes were resulted from mycorrhizal inoculation alone and this was observed at all growth periods as well as in both growing seasons. This increase likely to be due to the beneficial effect of mycorrhizal fungi as a result of P-supply increase by these fungi from insoluble phosphorus and consequently reflected on the actinomycetes proliferation. El - Ghandour (1992) found that VA- mycorrhizal infection had favourably effects on bacterial populations in the rhizosphere of mycorrhizal - infected plants compared with in the rhizosphere of non -infected ones.

Discarding the control, the lowest counts of actinomycetes were resulted from soil infested with *R. solani* either alone or in combination with *T. harzianum* and this result was true in both growing seasons. On the other hand, obtained results emphasize that the counts of actinomycetes were increased when mycorrhizal fungus (*G. macrocarpum*) was used in combination with either *R. solani or T. harzianum* as well as all of them. Also it is worthy to notice that the actinomycetes counts were higher in the 2nd season than in the 1st season in all treatments. This may be due to the difference in meterolgical factors (climatic conditions).

A-3. Periodical changes in fungi counts in the rhizosphere of volkameriana rootstock:

Data recorded in Table (3) show that total counts of fungi in the rhizosphere of volkameriana gradually decreased with the increasing of growth period and the highly decrease in the populations of fungi was observed in the treatments inoculated with either *G. macrocarpum* or *T. harzianum* alone. The heighest counts of fungi in the rhizosphere region were resulted from soil infested with *R. solani* alone as well as in the control treatment. This trend of results was observed in both seasons. Whereas, the lowest counts of fungi were resulted from either mycorrhizal or *T. harzianum* inoculation and this was true in both growing seasons. It is interested to notice that the inoculation with either *G. macarocarpus* or *T. harzianum* in combination with *R. solani* led to decrease the populations of fungi in rhizosphere zone. These results are in harmony with those obtained by Turner (1971), who reported that *T. harzianum* produce toxic substance gliotoxin (Tetracyclic compounds of piperazine and indole group, containing two sulfur atoms). This substance secreted by *T. harzianum* is a wide spectrum as anti-fungal substance. The growth inhibition of soil fungi by *T. harzianum* may be attributed to the rapid growth of *T. harziznum* and to the production of peptide antibiotics, acetaldehyde and acidic volatiles and phenol-like compounds (Dennis and Webster (1971) and Govindasamy and

Table (2): Periodical changes in actinomycetes counts (x 10^6 /g dry weight of soil) in the rhizosphere of volkameriana rootstock.

Treatments / Period (days)	Initial		30		60		90		120		150		180	
	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
Control	30	36	38	44	28	30	36	42	40.5	52	32	36.5	18.12	23
G. macrocarpum	41	48	60.2	74	72	80	102.5	106	120	132	92.8	98	80	88
T. harzianum	24	31	30	36	48	56	60.8	72.	80.6	84	56	60	24.8	26.2
Rhizoctonia solani	14	16	12.3	15.5	30.3	36	41.7	45	60.0	63	34.5	39.3	16	20.4
G. Macrocarpum + T. harzianum	30	38	48.2	54	40	56	60.3	82	70.1	78	80.4	84	38	42
G. Macrocarpum + R.solani	20	22	24.4	28	48	50	70	76	78	82	60.8	62	32	38
T. Harzianum + R. solani	10	12	16.3	19	21	25	28	35	48.2	54	50	52	20.6	22.3
G. Macrocarpum + T. harzianum + R. solani	20	24	28.2	30	40.5	48	78	82	86	95	90.4	96.6	52.5	60.8

Table (3): Periodical changes in total fungi counts (x 10^3 /g dry weight of soil) in the rhizosphere of volkameriana rootstock.

Period (days) / Treatments	Initial		30		60		90		120		150		180	
	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
Control	72	66	54	60	40	62	56	48	38	36	24	30	30	24
G. macrocarpum	8	6.2	3.2	1.6	4	3.2	2.2	1.2	1.4	2.6	3.2	2.6	1.3	1.4
T. harzianum	12	9.2	4	2	3.6	4.0	3.8	1.6	4.2	2.6	8.8	6.5	3.2	2.4
Rhizoctonia solani	120	100	90	82	60	80	42	40	30	24	22	26	20	18
G.macrocarpum + T. harzianum	34	29	18.2	20.5	10.6	8.4	20	24	12	10	.8	6	2.3	2.1
G. macrocarpum + R. solani	38	46	24	36	30	42	26	30	25	28	18	20	6.00	8.2
T. Harzianum + R. solani	44	41	30	33	20	28	14	18	8.6	8.2	6.3	8.4	2.5	2.8
G. macrocarpum + T. harzianum + R. solani	24	18	10.2	12.6	.5	8.00	6.8	4.6	2.4	2.6	2.0	4	1.2	1.6

Balasubramanian (1991)). In addition, Eisa *et al.*, (1994), reported that, the mycorrhizal fungi decrease or inhibit soil borne fungal diseases

B. Growth parameters and chemical analyses:

B-1. Effect of mycorrhizae, T. harzianum, R. solani and their combinations on growth parameters.

Results in Table (4) clearly show that seedlings inoculated with *G. macrocarpum* fungus alone gave the highest values of plant height, top and root dry weights as compared to the other treatments. Such results are in agreement with those obtained by Menge *et al.*, (1977), Edress (1982) and Gendiah *et al.*, (1991). Moreover, *T. harzianum* fungus induced an increase in values of top and root dry weights, while it reduced the plant height as compared to control plants. Similar results were obtained by Koehl and Schloesser (1989).

It is worthy to notice that, soil infestation with *R. solani* alone gave the lowest values of studied growth parameters and this result is in accordance with Wingfield (1968) and Eisa *et al.*, (1994).

Also, seedlings inoculated with *G. macrocarpum* and *T. harzianum* gave higher values of plant height, top and root dry weights than uninoculated ones (control) in both seasons. These results were confirmed with the findings by Koehl and Schloesser (1989).

Mycorrhizal fungus inoculation in combination with *R. solani* caused an increase in studied growth characters of Volkameriana as compared with soil infested with *R. solani* alone. These results are in harmony with those of Baltruschat and Schonbeck (1972); Ross and Marx (1972) and Baltruschat *et al.*, (1973). Also, Abdel-Fattah and Mankarios (1995) reported that addition of mycorrhizal fungus *Glomus mosseae* together with the pathogen fungus (*Chalara elegans*) significantly reduced the negative effect of the pathogen on Soybean growth characters.

On the other hand, the interaction between *T. harzianum* and *R. solani* fungi, remarked that the application of biological control agent with *R. solani* improved growth parameters since the plant height, top and dry weights increased as compared with *R. solani* alone. Similar results were also found by Koehl and Schloesser (1989).

Moreover, the addition of mycorrhzial and biological control fungi in combination with *R. solani* also increased plant height, top and root dry weights when compared with the application of either *T. harzianum* or *R. solani* each one alone. Similar results were reported by Koehl and Schloesser (1989).

Table (4): Effect of mycorrhizae fungus, *T. harzianum*, *R. solani* and the combination between them on growth of volkameriana seedlings.

Treatments	Plant height (cm)		Top dry weight g/plant		Root dry weight g/plant	
	1995	1996	1995	1996	1995	1996
Control	24.3bcd	34.7c	3.87ab	4.83bc	3.43bc	3.87bcd
G. macrocarpum	31.3a	44.7a	5.17a	8.00a	5.10a	5.17a
T. harzianum	22.3bcd	29.3d	3.18b	6.73a	3.40bc	4.73abc
Rhizoctonia solani	21.7d	28.7d	3.13b	3.62c	2.72c	2.67d
G. Macrocarpum + T. harzianum	29.0a	45.0a	4.17ab	5.83b	4.83b	5.07ab
G. Macrocarpum + R. solani	25.0bc	38.7b	3.57ab	4.80bc	3.62bc	4.07ab
T. Harzianum + R. solani	24.7bc	39.7b	4.63ab	5.50b	4.87a	4.00bcd
G. Macrocarpum + T. Harzianum + R. solani	25.7b	40.0b	3.93ab	5.27bc	4.17ab	3.57cd

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

B-2. Effect of mycorrhizae, T. harzianum, R. solani and their combinations on chlorophyll content.

Data presented in Table (5) indicate that mycorrhizae fungus alone gave significant increase in both chlorophyll a and b as compared with the analogous one of non - mycorrhizal plants and this result was confirmed with the findings of Gendiah (1987).

It is evident from Table (5) that chlorophyll contents (a or b and total) for inoculated plants with *T. harzianum* were significantly differed as compared to control plants. While, soil infestation with *R. solani* alone caused high reduction in values of chlorophyll a and b as compared with uninfested plants in both seasons. Similar results were found by Zaghloul and Abd El-Mageed (1996). Who reported that fungal infection greatly diminished the chlorophyll pigments content of faba bean plants.

Regarding the combination treatments, results clearly show that mycorrhizal inoculation in combination with *T. harzianum* gave significant increase in leaf chlorophyll contents as compared to soil inoculation with *T. harzianum* alone. The same trend of results was observed in the treatment included *G. macrocarpum* + *R. solani* since chlorophyll content was significantly increased as compared to soil infestation with *R. solani* alone. Also, soil inoculation with *T. harzianum and R. solani* improved leaf chlorophyll contents as compared to the using of *R. solani* alone.

Data in Table (5) also emphasize that, seedlings inoculated with *G. macrocarpum* + *T. harzianum* + *R. solani* gave significant increase in chlororphyll a and b as compared to soil infestation with *R. solani* alone and when was used combined with either *G. macrocarpum* or *T. harzianum* each one alone. This result clearly indicates that *G. macrocarpum* and *T. harzianum* can antagonize *R. solani* and consequently reduce its harmful effect.

B-3. Effect of mycorrhizae, T. harzianum, R. solani and their combinations on leaf macro-elements content.

Results in Table (6) reveal that seedlings inoculated with *G. macrocarpum* alone gave significant increase of leaf N, P, K and Mg content than uninoculated seedlings. While, leaf Ca values did not reach to the significant level between mycorrhizal and non-mycorrhizal plants in the first season. These results are in accordance with the findings of Menge *et al.*, (1980), Edrees (1982) and Gendiah (1987). On the other hand, soil inoculated with *T. harzianum* alone had no significant effect on leaf N, P, K and Mg contents of volkameriana seedlings in both seasons. Except leaf P and K contents in the second season and Ca content in the first season, seedlings inoculated with *T. harzianum* exhibited similar values of macro-elements content as compared to control plants. Similar results were obtained by Husien (1996).

Table (5): Effect of mycorrhizae fungus, *T. harzianum*, R. solani and the combination between them on leaf chlorophyll content of volkameriana seedlings.

Treatments	1995			1996		
	Chlorophyll			Chlorophyll		
	a	b	Total	a	b	Total
Control	78.8bc	56.0cd	134.8bc	76.2c	58.0c	134.2cd
G. macrocarpum	96.3a	75.3a	171.6a	99.9a	78.3a	178.2a
T.harzianum	75.0cd	57.0bc	132.0c	77.8bc	58.8c	136.6c
Rhizoctonia solani	23.0f	11.2f	34.2f	21.2e	11.8e	33.0f
G.macrocarpum+T. harzianum	92.8a	79.2a	178.0a	102.2a	81.8a	184.0a
G.macrocarpum+R. solani	71.0d	52.0de	123.0d	74.2c	54.2c	128.4d
T.harzianum+R. solani	58.0e	48.0e	106.0e	61.0d	50.2d	111.2e
G.macrocarpum+T.harzianum+R. solani	81.8b	60.8b	142.6b	82.3b	65.3b	147.6b

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

Table (6): Effect of mycorrhizae fungus, *T. harzianum, R. solani* and the combination between them on leaf macro-elements content of volkameriana seedlings.

Treatments	N%		P%		K%		Ca %		Mg %	
	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
Control	1.60c	2.0bc	0.19c	0.20b	1.20c	1.60bc	3.00b	3.80b	0.72cd	0.96cd
G. macrocarpum	2.60a	2.40a	0.24ab	0.23a	1.80a	2.20a	3.00b	4.20a	1.32b	1.24b
T. harzianum	1.60c	1.80cd	0.19c	0.15c	1.34bc	1.20de	3.40a	4.00ab	0.96c	1.08bc
Rhizoctonia solani	1.50c	1.60d	0.16d	0.13c	0.90d	1.00ef	2.60c	3.00d	0.60d	0.72e
G. Macrocarpum + T. harzianum	2.00b	2.20ab	0.23ab	0.21ab	0.86d	0.90f	2.20d	2.40e	1.32b	1.08bc
G. Macrocarpum + R. solani	1.60c	2.00bc	0.17cd	0.15c	1.40bc	1.40cd	3.00b	4.00ab	0.72cd	0.96cd
T. Harzianum + R. solani	1.60c	2.00c	0.22b	0.20b	1.28c	1.20de	2.80bc	3.80b	0.84c	0.84de
G. Macrocarpum + T. Harzianum + R. solani	2.00b	2.00bc	0.25a	0.21ab	1.56ab	1.80b	2.80bc	3.40c	1.60a	1.84a

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

Data in Table (6) also show that soil infestation with *R. solani* alone gave lower values of leaf N, P, K, Ca and Mg contents as compared to control. The differences between diseased and healthy plants (control) reached to the significant level in leaf N in both seasons and P in the second season, but it did statistically had no significant effect in leaf K, Ca, and Mg contents. These results are in agreement with those of Wingfield (1968) Baltruschat and Schonbeck (1972) and Husien (1996).

Results in Table (6) also reveale that seedlings inoculated with *G. macrocarpum* + *T. harzianum* fungi gave higher values of leaf N, P, and Mg contents than either control plants or seedlings inoculated with *T. harzianum* fungus alone. The opposite was true in leaf K and Ca contents. Also, mycorrhizae treatment alone gave the highest values of leaf N, P, K and Mg contents in both seasons as compared with other treatments under study. The same effect in increasing leaf Ca content was observed in seedlings inoculated with *T. harzianum* fungus alone. Similar results were found by Edrees (1982), Gendiah (1987) and Husien (1996).

On the other hand, seedlings inoculated with *G. macrocarpum* + *R. solani* fungi gave a slight increase in leaf N, P, K and Mg contents than seedlings inoculated with *R. solani* fungus alone. The increment reached to the significant level in leaf K and Ca (in both seasons), N and Mg (in the second season) contents. However, combined treatment gave the same values of leaf N, P, K, Ca and Mg contents as compared with control plants. These results were reported by Husien (1996).

Regarding the combination between *T.harzianum* and *R. solani* fungi, seedlings inoculated with both fungi gave higher values of leaf N, P, K, Ca and Mg contents than seedlings inoculated with *R. solani* fungus alone. Also, combined treatment was more effective in increasing leaf, P content than seedlings inoculated with *T. harzianum* fungus alone, but N, P, K, Ca and Mg values did not reach to the significant level. The lowest values of leaf, N, P, K, Ca and Mg contents were noticed in diseased seedlings with *R.solani* fungus. Generally, biological control fungus succeeded in reaching diseased plants with *R.solani* fungus to control plants in leaf, N, P, K, Ca and Mg contents.

On the other hand, seedlings inoculated with G. *macrocarpum* + *T. harzianum* + *R. solani* fungi gave higher values of leaf, N, P, K, Ca and Mg contents than these treated with *R.solani* fungus or control plants. Also, combined treatment with the three fungi surpassed all other treatments in leaf, P and Mg contents, but it had the second rank in leaf, N, K and Ca contents.

B-4. Effect of mycorrhizae, T. harzianum, R. solani and their combinations on root macro-elements content.

Data in Table (7) indicate that mycorrhizal inoculation alone gave the highest values of N, P, K and Ca in roots of volkameriana seedlings and this was

true in both growing season. These results are in line with those reported by Gendiah (1987), Abd El-fattah and Mankarios (1996).

The addition of *T. harzianum* to the soil had no significant effect on nitrogen and phosphorus contents in both seasons whereas, the addition of *T. harzianum* had significant effect on potassium and calsium contents as compared to control plants.

Results in Table (7) also reveal that soil infestation with *R. solani* alone gave significant decrease in root, N, P, and Ca contents of volkameriana plants as compared with control plants. In contrast, root K, and Mg contents were significantly increased.

Data in Table (7) also indicate that seedlings inoculated with *G. macorcarpus* + *T. harzianum* fungi gave similar values in root N, P and K contents as compared with control plants, but combined treatment reduced root Ca and Mg contents. However, mycorrhizal fungus plants had a higher root N, P, K, Ca and Mg contents than combined treatment, while, root N, P, K and Ca values of T. harzianum treatment were more or less similar in values of root seedlings inoculated with two fungi (*G. macorcarpus* + *T. harzianum*).

On the other hand, the addition of mycorrhizae fungus in combination with *R. solan* induced an increase in root N, K, and Mg contents than plants inoculated with *R. solani* fungus alone, but root P and Ca contents of infected plants did not affect by mycorrhizal inoculation. Also, mycorrhizal inoculation succeeded in reaching diseased seedlings with *R. solani* fungus to control plants in root N (in both seasons) and p (in the first season) contents. But failed in root P (in the second season), Ca and Mg contents in both seasons. Meanwhile, combined treatment increased root K content than control plants.

However, *T. harzianum* + *R. solani* fungi treatment reduced root N and P contents than control plants, while, combined treatment increased root K content, but root Ca and Mg contents had not affected. Also, combined treatment increased root N, P, K and Ca contents of seedlings inoculated with *R. solani* fungus, but the contrast was true in root Mg content. *G. macrocarpum* + *T. harzianum* + *R. solani* fungi treatment gave similar values of root N and P contents as compared with control plants while, root Ca and Mg contents decreased. However, combined treatment increased root K content than control plants. Generally, mycorrhizal plants gave the highest root, N, P, K and Ca contents. This trend was not observed in root Mg content. Similar results were reported by Gendiah (1987) and Bellone and deBellone (1993).

II- Infection percentage and colonization intensity of mycorrhizal fungus in volkameriana roots as influenced by *T. harzianum* and *R. solani* fungi:

Data presented in Table (8) clearly show that the infection percentage with *G. macrocarpum* fungus for volkameriana roots increased and reached to

Table (7): Effect of mycorrhizae fungus, *T. harzianum*, *R. solani* and the combination between them on root macro-elements content of volkameriana seedlings.

Treatments	N %		P %		K %		Ca %		Mg %	
	1995	1996	1995	1996	1995	1996	1995	1996	1995	1996
Control	1.60cd	2.20b	0.16ab	0.18bc	1.20c	1.30c	2.80ab	2.60b	1.32cd	1.48b
G. macrocarpum	2.30a	2.60a	0.17a	0.23a	1.70a	2.40a	3.00a	3.20a	1.08d	1.20c
T. harzianum	1.80bc	2.00b	0.16ab	0.19b	1.40bc	2.20a	2.60bc	2.00de	2.16a	1.92a
Rhizoctonia solani	1.40d	1.50c	0.15bc	0.14f	1.28c	1.80b	2.20d	2.00de	1.80b	2.02a
G. macrocarpum+T. harzianum	2.00b	2.20b	0.16ab	0.17cd	1.20c	1.20b	2.40cd	2.20cd	0.60e	0.84d
G. Macrocarpum + R. solani	1.60cd	2.10b	0.15bc	0.15ef	1.60ab	1.60b	1.80e	1.80e	0.48e	0.72d
T. Harzianum + R. solani	1.60cd	1.60c	0.14c	0.16de	1.40bc	1.60bc	2.60bc	2.40bc	1.48c	1.60b
G. Macrocarpum + T. Harzianum + R. solani	1.60cd	1.70c	0.17a	0.19b	1.80a	2.20a	1.80e	2.20cd	1.24d	1.32c

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

Table (8): Infection percentage and colonization intensity of mycorrhizal fungus in volkameriana roots as influenced by *T. harzianum* and *R. solani* fungi in 1995 and 1996 seasons

Treatments	Infection %		Average number					
			Vesicles		Arbuscules		Mycelium	
	1995	1996	1995	1996	1995	1996	1995	1996
Control	0.0b	0.0b	0.0d	0.0d	0.0d	0.0d	0.0d	0.0d
G. macrocarpum	100a	100a	39.0b	42.0b	15.0b	17.0b	2.0bc	3.0b
T. harzianum	0.0b	0.0b	0.0d	0.0d	0.0d	0.0d	0.0d	0.0d
Rhizoctonia solani	0.0b	0.0b	0.0d	0.0d	0.0d	0.0d	0.0d	0.0d
G. macrocarpum+T.harzianum	100a	100a	38.0b	38.0b	12.0b	14.0b	3.0b	3.0b
G. macrocarpum + R. solani	100a	100a	16.0c	17.0c	7.0c	7.0c	1.0c	1.0c
T. harzianum + R. solani	0.0b	0.0b	0.0d	0.0d	0.0d	0.0d	0.0d	0.0d
G. Macrocarpum + T. Harzianum + R. solani	100a	100a	56.0a	72.0a	32.0a	37.0a	5.0a	5.0a

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

the maximum level (100 %) in the treatments inoculated with mycorrhizae fungus either alone or in combination with *T. harzianum* and /or *R. solani*. The same trend was reported by Gendiah *et al.*, (1991) and Husien (1996). Nevertheless, root infection percentage of seedlings without mycorrhizae fungus either treated or untreated with *T. harzianum* and *R. solani* fungi was nil.

As regard to the average number of vesicles, arbuscules and mycelium in different treatments inoculated with mycorrhizae fungus, obtained data show that the average number of vesicles, arbuscules and mycelium reached maximum values in plants treated with *G. macrocarpum* + *T. harzianum* + *R. solani* fungi as compared with other mycorrhizal treatments. Also, either *G. macrocarpum* + *T. harzianum* fungi or *G. macrocarpum* alone treatments gave the second rank in increasing vesicles, arbuscules and mycelium numbers. In other words, vesicles, arbuscules and mycelium intensity of *G. macrocarpum* fungi were not affected by *T. harzianum* fungus. While the presence of *R. solani* fungus seams to be a barrier to the intensity of *G. macrocarpum* fungus. Such results were found by Baltruschat and Schonbeck (1972) and Koehl and Schloesser (1989).

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تأثير التسميد الحيوى و المقاومه الحيويه على النمو و التركيب الكيميائى لشتلات الفولكاماريانا

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- أجرى هذا البحث بكلية الزراعة بمشتهر خلال موسمى ١٩٩٥،١٩٩٦ لدراسة تأثير التسميد الحيوى والمقاومة الحيوية على نمو شتلات الفولكاماريانا كأصل هام من أصول الموالح فى مصر. ولقد استخدم فى هذه الدراسه فطر الميكورهيزا *G.macrocarpum*، فطر *T. harzianum* وفطر *R.solani* كمسبب لمرض عفن الجذور وذبول البادرات فى معظم المحاصيل. ويمكن تلخيص أهم نتائج هذه الدراسه فيما يلى :-
- أوضحت النتائج أن العدد الكلى للبكتيريا و الأكتينوميسيتات فى منطقة الريزوسفير ازدادت بزيادة فترة النمو حيث شوهدت أعلى الأعداد فى الفترة ما بين ٩٠-١٢٠ يوم وقلت بعد ذلك تدريجيا.

- بالنسبه للعدد الكلى للفطريات فلقد أوضحت النتائج أن أعداد الفطريات قلت فى منطقة الريزوسفير بزيادة فترة النمو مع كل المعاملات.
- أعلى أعداد للبكتيريا شوهدت عند المعاملة بفطر الميكورهيزا + فطر الترايكودرما + فطر الرايزوكتونيا بينما أعلى أعداد للأكتينوميسيتات شوهدت عند المعاملة بفطر الميكورهيزا بمفرده.
- أعلى أعداد للفطريات لوحظت عند المعاملة بفطر الرايزوكتونيا سولانى بمفردة وكذلك الكنترول.
- أدى التلقيح بفطر الميكورهيزا أو الترايكودرما أو كلاهما معًا الى نقص حاد فى أعداد فطريات التربة .
- عند تلقيح التربة بفطر الميكورهيزا و الترايكودرما فى وجود أو عدم وجود فطر الرايزوكتونيا شوهد زيادة معنوية فى أطوال النباتات و الأوزان الجافه لكل من المجموع الجذرى والخضرى وكنلك محتوى الأوراق من الكلوروفيلات.
- أوضح التحليل الأحصائى للنتائج أن التلقيح بفطر الميكورهيزا أدى الى زيادة محتوى الأوراق والجذور من النيتروجين و الفوسفور والبوتاسيوم والماغنسيوم والكالسيوم مقارنة بالنباتات الغيرملقحة بفطر الميكورهيزا.
- عند المعاملة بفطر الرايزوكتونيا سولانى بمفرده وجد أن محتوى الأوراق والجذور من العناصر الغذائية السابقه قد قل معنويا.
- عموما أوضحت النتائج أن التلقيح بفطر الميكورهيزا + فطر الترايكودرما فى وجود فطر الريزوكتونيا قد حسّن من محتوى الأوراق والجذور من العناصر الغذائية وذلك بالمقارنة بالتلقيح بفطر الميكورهيزا أو فطر الترايكودرما كل بمفرده فى وجود فطر الرايزوكتونيا سولانى.

أوضحت النتائج أن أعلى نسبة منوية للإصابة بالميكورهيزا شوهدت عند المعاملة بفطر الميكورهيزا + فطر الترايكودرما + فطر الرايزوكتونيا وكذلك أعطت نفس المعاملة أعلى الأعداد من جراثيم و ميسليومات الميكورهيزا.

عموما أوضحت النتائج المتحصل عليها أن التلقيح بفطر الميكورهيزا سواء بمفرده أو مختلطا مع فطر الترايكودرما فى وجود فطر الريزوكتونيا سولانى أدى الى تحسين صفات النمو و التركيب الكيميائى لنباتات الفولكاماريانا. ويمكن أن يعزى هذا التأثير الإيجابى الى مقدرة فطر الميكورهيزا والترايكودرما على تثبيط فطر الريزوكتونيا سولانى وهذا ينعكس على تقليل أثره الضار.