Studies on growth, nutritional and microbiological status of citrus seedlings infested with root-rot disease

1Elham Z. Abd El-Motty; 2 Selim, El-Metwally; 2 Youssef, Rifaat Abou and 3 Sahab, Ahmed Farahat.
1 Pomology Dept., National Research Centre, Giza, Egypt
2 Soils and Water Use Dept., National Research Centre, Giza, Egypt.
3 Plant Pathology Dept., National Research Centre, Giza, Egypt.
ahmedsahab2002@yahoo.co.uk

Abstract: This research aims to evaluate the suppressive effects of compost fortified with Trichoderma harzianum and Top.Zn formulations on citrus root-rot and plant growth. Pathogenicity test proved that isolate no.1 of Fusarium solani and Macrophomina phaseolina were the most frequently causing infection of all orange plants with 87.5 and 93.75% disease severity respectively. Soil infested with F. solani or M. phaseolinea decreased plant growth and N, P and K contents in the orange leaf tissues compared to the control. Meanwhile, application of Top.Zn compound alone raised up N, P and K contents (%) in leaves of orange and mandarin survived in soil infested with F. solani and M. phaseolina. Use of compost with T. harzianum and Top.Zn simultaneously with a pathogen inoculation caused a significant increase in plant growth, chlorophyll a and b, macronutrients (N, P and K) content, micronutrient (Fe, Zn, Mn and Cu) contents orange and mandarin seedlings. The total fungal and bacterial counts in the orange and mandarin rhizosphere were increased progressively as the plant grew up reaching their maximum at the last count which was taken after 90 days (seedlings were 1-year old). In soil infested with F. solani and M. phaseolina, treatment with compost fortified with T. harzianum increased the total fungal count 3.34 and 28.98 times, respectively in orange and 2.60 and 21.99 times, respectively in mandarin compared with non treated control. In soil infested with F. solani and M. phaseolina, the treatment with compost fortified with T. harzianum in combination with Top.Zn decreased the average number of total bacterial counts in the rhizosphere of orange 85.04 and 78.92% respectively and 59.32 and 92.74 % respectively in the rhizosphere of mandarin. [Nature and Science. 2010;8(4):112-121]. (ISSN: 1545-0740).

Key words: Citrus root rot, rhizosphere, compost, Trichoderma harzianum, Top.Zn formulation.

1. Introduction

Citrus root-rot caused by *Fusarium solani* (Mart) Snyd & Hans and *Macrophomina phaseolina* was reported to attack citrus varieties (Kore and Mane 1992; Rensburg, *et al.* 2001; Kung'u *et al.* 2002; El-Mohamedy and Ahmed 2009). Although root-rot is under control due to (Verma *et al.* 1999) the pressure against the use of fungicides due to high coast, fear of resistance and potential hazards to the environment have resulted in a shift towards biological control using compost fortified with antagonistic microorganisms could be the way to control root-rot citrus disease.

Compost applied to the soil improves its quality by altering the chemical and physical properties, increase organic matter content, water holding capacity, overall diversity of microbes, provide macro-and micronutrients for plant growth and suppress diseases which indirectly contribute to plant growth enhancement (Scheuerell and Mahaffe 2004; Sylvia 2004; Healther *et al* 2006).

Certain microorganisms present in the compost such as *Trichoderma* spp. are known to stimulate plant growth (Ozbay and Newman 2004; Sylvia, 2004). These microbes benefit for the plant through different mechanisms action, including the production of secondary metabolities, antibiotics and hormone like substances (Ozbay and Newman 2004; Harman *et al.* 1996). The production of siderophores, antagonistic to soil borne root pathogens (Dubeikovsky *et al.* 1993; Siddiqui *et al.* 2008) has been also reported.

The bio-efficiency of compost therefore, could also be further enhanced by fortifying it with plant nutrients or biocontrol inoculants such as *Trichoderma* spp. *Trichoderma harzianum* alone or in combination with compost has been documented as the most common and effective biocontrol agent for disease control in various host-pathogen systems (Elad 2000; Ibrahim 2005; Siddiqui *et al.* 2008).

Therefore, this study was carried out to determine the efficiency of compost fortified with *T. harzianum* as an alternative to chemical fungicide and Top.Znformulation on morpho-physical growth and occurrence of root rot disease of orange and mandarin citrus seedlings. The effect of different treatments on rhizosphere soil microflora was also studied.

2. Material and Methods:

Pathogens isolation and identification

Since 2006, thirty diseased citrus seedlings (one-two years old) grown under greenhouse conditions in Behera and El-Giza Governorates were sent to the laboratory of National Research Centre (NRC), Egypt, to identify the organisms causing root-rot disease. Selected root segments of diseased plants were washed by running water for one hours and surface disinfected by immersion in a 0.5% sodium hypochlorite solution for 2 min, rinsed with sterilized water, dried under a laminar airflow hood, and cut into pieces (3-5 mm). The root pieces were placed on plates containing potato dextrose agar (PDA) for 3-7 days at 28°C±2. Fungal isolates were purified using hyphal tip and single spore culture techniques (Booth 1971).

Identification of pure culture was carried out according to Gilman (1957), Booth (1971), Nelson *et al* (1983), Barnett and Hunter (1986).

Pathogenicity tests

Autoclaved oat (100g oat / 70 cm^3 water) was infected with plugs of a 10 days-old pure culture of each isolated fungi and incubated for 2-3 weeks. Sterilized oat grains without fungi were used as control. Soil was infested with the rate of 10% (w/w), watered every two days for one week before planting. One transplant (1 year old) of citrus orange seedlings (cv. valincia) was transplanted in each pot. Four pots were used for each isolate as replicates. Four months after planting disease symptoms were registered daily on shoot system as follows: 0 = healthy, 25% = yellowish, 50% = plant wilted, 75% = whole plant wilted and 100% = plant dead showed severe wilt. When foliar symptoms appeared, rotted root segments were surface disinfected as described before and placed on PDA plates for reisolation of the pathogen.

Compost (farmyard manure)

One year old farmyard manure produced by NRC, Giza, Egypt was used and analyzed. The chemical properties were determined using the method described by A.O.A.C. (1990). The chemical analyses are summarized in table 1.

Table 1. Chemical characteristics of the compost (farmyard manure)

pH	EC(dS/m)	C%	N%	C/N ratio	Total P%	Total K%	OM%
6.90	4.30	17.60	1.18	14.92	0.41	1.07	26.38

A representative soil sample taken from the used soil was analyzed and listed in table 2.

Table 2. Some physical and chemical properties of the soil used in the experiment.

Particle size distribution [%]			Texture soil	EC dS/m	PH	Available nutrients [mg/]						
Sand	Silt	Clay				N	P	K	Fe	Mn	Zn	Cu
41.18	30.95	27.87	Clay loam	2.3	7.8	30	10	286	5.8	4.7	0.9	0.31

Compost fortified with biocontrol agent

The highly antagonistic isolate of *T. harzianum* obtained from previous work by Haggag and Saber (2000) exhibited a broad-range of antibiosis towards many plant pathogens was grown on PDA broth medium in 500 ml capacity conical flask for 28°C±2. Conidia spores and mycelial growth were harvested to obtained fungal suspension of 3x 10^8cfu/ml. Farmyard manure compost was inoculated with 10 ml/ 100g on oven dry basis of *T. harzianum* spore suspension and inoculated in plastic containers in the dark at room temperature (25-28°C). **Top. Zn formulation**. Top.Zn (8 hydroxy quinoline sulphate) was kindly obtained from soil and water use Dept. NRC, Egypt.

Greenhouse experiments

During two consecutive seasons (2008-2009) a pot experiment was carried out to evaluate the role of compost fortified with *T. harzianum* and Top.Zn at rate of kg/600L against *F. solani* and *M. phaseolina* the causal agents of root-rot disease of orange cv. valincia and mandarin cv. Egyptian balady citrus plants. Each pots containing 5kg soil somewhat wet (about 60% water holding capacity) were inoculated with a propagules of *F. solani* and *M. phaseolina* at a rate of 50g/pot, 3days before cultivation to ensure the distribution of the inocula. Four orange and mandarin seedlings one year old were used as a replicates for each treatment. Untreated seedlings were used as a control. *F. solani* and *M. phaseolina* prepared as mentioned in the pathogenicity tests were used.

On the other hand, bioagent was used at the rate of 50g/pot by drenching soil with compost individual and in combined treatments to soil before transplanting citrus seedlings.

Treatments were categories for each citrus varieties into the following:

1-Un-infested soil (control).
2-Infested with *F. solani*
3-*M. phaseolina*
4-*T. harzianum*
5-*F. solani* + *T. harzianum*
6-*M. phaseolina* + *T. harzianum*
7-*F. solani* + Top.Zn
8-*M. phaseolina* + Top.Zn
9-*F. solani* + *T. harzianum* + Top.Zn
10-*M. phaseolina* + *T. harzianum* + Top.Zn

Plant growth analysis On the late of September, a leaf sample consisted of 50 leaves was taken from the mid shoots transplant according to Jones *et al*. (1991). Plant height (cm), branch length (cm), stem thickness at 5 cm above the crown was measured by gauge (mm) and leaf area using Li- 3100 area meter were recorded. Number of mature leaves was counted on each seedling.

Chemical characteristics

a- Chlorophyll (a and b)

Chlorophyll a and chlorophyll b content were determined using spectrophotometer at a wave length, 647 and 664 nm proposed by Coombs *et al.* (1987).

b- Macro and Micronutrients analysis

To analyze macro and micronutrients in citrus, samples were taken from each treatment, then dried at 70°C, and it was grounded using stainless steel equipments. From each sample 0.2 g was digested using 5 cm^3 of the mixture of sulfuric (H_2SO_4) and perchloric ($HCLO_4$) acids (1:1) as described by Peterburgski (1968). Total nitrogen was determined by micro-Kjeldahl method and phosphorus was determined calorimetrically at wavelength 680 nm using spectrophotometer (Spekol) as well as potassium was determined by using Gallen Kamp flame photometer. Micronutrients, i.e., Zn, Fe, Mn and Cu were measured using atomic absorption spectrophotometer Perkin Elmer model 5000 (Cottenie *et al.* 1982).

Microbiological analysis

The total bacterial and fungal counts in the rhizosphere of the two citrus varieties were obtained from each treatment after 30, 60 and 90 days. The method adopted by Louw and Webely (1959) for studying the microorganisms of rhizosphere soil region was used. A portion of root system was taken with great care to obtain soil very closed to root-system as much as possible and transferred to a wide mouth reagent bottle of known weight containing 90 cm^3 distilled water under aseptic conditions. The plate count technique according to Allen (1961) was followed for total count of bacteria and fungi. Soil extract agar medium (Buent and Rovira 1955) and dilutions of $1/10^5$ to $1/10^7$ were used for bacteria and incubated at 35°C±2 for 48 hrs. Martin medium (Allen 1961) was used for fungi at dilutions of $1/10^3$ to $1/10^5$. Plates were incubated at 28°C±2 for 5-7 days.

Statistical analysis

Data of the present study were statistically analyzed and the differences between the means of the treatments were considered significantly when they were more than least significant differences (LSD) at the confidence level of 5% as outlined by Duncan (1955).

3. Result Analysis

Root-rot pathogens of citrus seedlings

Isolation from rotted roots seedlings revealed the association of one or more of the following six fungal species, i.e., *Alternaria tenuis*, *Chaetomium globosum, Fusarium solani*, *Macrophomina phaseolina*, and *Rhizoctonia solani***.** *F. solani* and *M. phaseolina* in particular were more frequent than any of the other fungi. These fungi were previously reported to be associated with root-rot of citrus in other countries (Kore and Mane 1992, Rensburg *et al*. 2001, Kung'u *et al.* 2002). In Egypt, El-Mohamedy and Ahmed (2009) reported that dry root-rot disease of mandarin caused by *F. solani* attack most citrus varieties.

Upon testing the pathogenicity of these isolated fungi, isolates of *Fusarium* and *Macrophomina* were found more or less able to attack orange citrus seedlings (Table 3). Orange seedlings were highly vulnerable to attack by the all three isolates of *F. solani* and the two isolates of *M. phaseolina*. The isolates no. 1 of *F. solani* and *M. phaseolina* caused 100% plant infection causing 87.5 and 93.75% disease severity on plant growth of orange and mandarin, respectively. *F. solani* and *M. phaseolina* have been known to be the main organisms causing root-rot of citrus (Strauss and Labuschagen 1999, Catara and Polizza 1999).

Table 3. Disease incidence on one year seedlings of citrus (orange cv.) artificially inoculated with isolates representing fungal species associated with root-rot disease and their severity on plant

Tested fungal Isolates	Isolate No.	Plants infected [%]	Severity on plant growth [%]
Alternaria tenuis		0	-
Chaetomium globosum		0	-
Fusarium solani	1	100	87.5
Fusarium solani	2	50	25.0
Fusarium solani	3	75	25.0
Macrophominea phaseolinea	1	100	93.75
M. phaseolinea	2	50	25.0
Rhizoctonia solani	1	25	6.25
R. solani	2	50	12.50
R. solani	3	50	31.25
LS D (0.05)		18.77	

Effect of compost fortified with *T. harzianum* and TopZn

1. plant growth parameters

It could be noticed from data presented in Table (4) that orange or mandarin seedlings which survived in infested soil with *F. solani* and *M. phaseolina* never attained the normal growth in height or other growth parameters. The stem height of orange seedlings survived in *F. solani* and *M. phaseolina* infested soil reduced more 3.66 and 11.69% respectively compared to the control. A similar trend was observed with the other growth parameters.

Data also showed that different growth parameters of orange and mandarin seedlings were significantly increased over those of seedlings growing in control pots (untreated) and also seedlings growing in soil infested with the root-rot pathogens (*F. solani* and *M. phaseolina).* The increment in seedlings height, branch length, number of branches and leaves, stem diameter and leaf surface area were increased significantly in seedlings treated with compost fortified with *T. harzianum* alone or together with *F. solani* and *M. phaseolina.* Possible explanations of this phenomenon include: control of pathogens leading to stronger growth and nutrient uptake (Ousley *et al.* 1993), solubilization of insoluble micro nutrients in soil (Altomare *et al.* 1999), and production of growth hormones (Windham *et al.* 1986).

In addition treatment of orange and mandarin seedlings infested with *F. solani* and *M. phaseolina* with Top.Zn alone or in combination with compost fortified with *T. harzianum* exhibited a highly significant effect. The stem height of orange and mandarin seedlings treated with compost fortified with *T. harzianum* alone was increased from 45.67 to 64.67 cm (41.60% increase) and from 39.67 to 58.67 cm (47.89% increase) respectively. The same trends were also observed with the other growth characters. Data also showed that orange plants survived in soil infested with *F. solani* and *M. phaseolina*, used fertilized compost fortified with *T. harzianum* + Top.Zn increased the number of leaves orange from 15.00 to 26.67 (77.8%) and from 13.00 to 26.33 (102.54%) respectively for both pathogens, while on mandarin plants the number of leaves was increased 83.36% and 111.08 % respectively for both pathogens.

Chlorophyll a and b contents recorded in citrus leaves infected with *F. solani* and *M. phaseolina* were lower than in the uninfected control. In addition treatment of orange and mandarin seedlings infested with *F. solani* and *M. phaseolinea* with Top.Zn alone or in combination with compost fortified with *T. harzianum* exhibited a highly significant effect. Since, chlorophyll a in the leaves of orange seedlings treated with Top. Zn and compost fortified with *T. harzianum* alone was increased from 73.67 to 89.38 (21.32% increase) and from 73.67 to 87.44 (18.69% increase) in soil infested with *F. solani* and *M. phaseolina* respectively. As reported by several investigators that compost made of agricultural and industrial wastes have been widely used as soil amendment and induced suppression of soil borne pathogen through biological mechanisms (De Couster and Hoitink 1999; Muhammed and Amusa 2003; Rivera *et al*. 2004)**.**

Table 4. Effect of compost fortified with *T. harzianm* and Top.Zn on the growth parameters of two citrus seedlings infected with *F. solani* and *M. phaseolina*

Growth parameters / Treatments	Untreated	Soil infested with									
		T. harz	***F. solani***				***M. phaseolina***				
			Infes.	***T. harz.***	**Top.Zn**	**Top.Zn.+ *T. harz***	**Infes.**	***T. harz.***	**Top.Zn**	Top.Zn + ***T. harz***
Orange											
Stem length [cm]	* 45.67g	64.67a	44.00h	57.00d	52.33ef	62.33b	40.33f	53.33e	51.00f	60.33c
Branch length [cm]	6.27g	10.17a	5.67h	8.60d	70.17f	9.60b	5.40h	8.13e	6.50g	9.10c
No. of branches	2.00bc	4.00a	1.00c	2.00bc	1000bc	3.00ab	1.00c	2.00bc	2.67abc	3.00ab
No. of leaves	18.33f	28.67a	15.00g	24.33c	2.67e	26.67b	13.00h	22.33d	20.00a	26.33b
Main stem diam. [cm]	0.73d	1.17a	0.54gh	0.71de	0.63ef	0.92b	0.51h	0.65ef	0.61fg	0.83c
Branch diam. [cm]	0.60c	0.80a	0.53d	0.64c	0.62e	0.74b	0.52d	0.63c	0.61e	0.65c
Leave area [cm]	28.74g	40.06a	27.12h	33.42d	29.59f	37.80b	26.40h	31.39e	29.11g	35.41c
Chlorophyl a	73.67h	91.03a	70.28f	58.34d	78.42f	89.38b	68.47f	80.38e	75.47g	87.44c
Chlorophyl b	27.40f	33.32a	26.17g	28.34d	27.45ef	30.35b	25.33h	28.23de	27.57def	29.25c
Mandarin											
Stem length [cm]	39.67g	58.67a	38.00h	51.00d	46.33ef	56.33b	34.33f	47.33e	45.00f	53.67c
Branch length [cm]	6.10e	9.13a	5.33f	8.20c	6.17e	8.60b	5.17f	7.10d	5.50f	8.10c
No. of branches	1067cd	3.00a	1.00d	1.00d	1.67cd	2.67ab	2.00bc	1.00d	2.33abc	2.67ab
No. of leaves	17.33f	27.67a	14.00g	23.33c	19.67e	25.67b	12.00h	21.33d	19.00e	25.33b
Main stem diam. [cm]	0.63d	1.1a	0.44f	0.61d	0.53e	0.82b	0.41f	0.55e	0.51e	0.73c
Branch diam. [cm]	0.50c	0.70a	0.43d	0.54c	0.52e	0.64b	0.42d	0.53c	0.51e	0.55c
Leave area [cm]	27.74g	39.06a	26.12h	32.42d	28.95f	36.00b	25.40h	30.39e	28.11g	34.41c
Chlorophyl a	63.67h	90.03a	60.28e	75.34d	68.42e	79.38b	60.47f	79.38b	65.47g	77.44c
Chlorophyl b	26.40e	31.31a	25.17f	27.33d	26.42e	29.35b	24.33g	27.23d	26.54e	28.25c

*Means values followed by the same letter within the treatments are not significantly different ($p < 0.05$) according to the Duncan's multiple range tests

2. Nutritional status

Data in Table 5 show the effect of soil treatment with compost fortified with *T. harzianum* and Top.Zn on leaf of micro- and macronutrients of orange and mandarin seedlings. Compost fortified with *T. harzianum* and Top.Zn applications had a significant effect on nutrient contents (%) in orange and mandarin citrus leaves (*p< 0.05).* Soil infested with *F. solani or M. phaseolina* decreased N, P and K content in the orange leaves compared to the control. Opposite trend was observed in mandarin leaves. Data also indicate that soil infested with compost fortified with *T. harzianum* alone improved N content in orange leaves while, P and K contents were decreased compared with untreated control. On the contrast, the same treatment improved the macronutrients, i. e., N, P and K in the leaves of mandarin.

Application of Top.Zn compound alone raised up the macronutrients in leaves of orange and mandarin survived in infested soil with *F. solani* and *M. phaseolina*. As, the percentage of N, P and K in orange leaves infested with *F. solani* were 3.35, 0.236 and 1.88%, respectively compared with 1.66, 0.214 and 1.72% respectively in the control. The same trends were also observed in leaves of orange and mandarin when soil was treated with Top.Zn + compost fortified with *T. harzianum*

Concerning micronutrients in the leaves, results cleared that soil infested with the two root-rot pathogens reduced Fe, Zn, Mn and Cu in orange leaves , while in mandarin leaves only Fe content was decreased comparing to the control. Application of Top.Zn alone or in combination with compost fortiied with T. *harzianum* also raised up the micronutrients percentage in leaves of orange and mandarin survived in soil infested with *F. solani* and *M. phaseolina* respectively. Possible explanations of this phenomenon include: control of minor pathogens leading to stronger growth and nutrients uptake (Ousley *et al.* 1993) solubilization of insoluble minor nutrients in soil (Altomare *et al.*, 1999) and production of growth hormones (Windham *et al.* 1986). *Trichoderma harzianum* may enhance plant growth by increasing the solubility of zinc, copper, iron and manganese ions, all plant nutrients with low solubility (Yedidia *et al.* 2001). Altomare *et al.* 1999 reported that *T. harzianum* increases plant nitrogen efficiency and also solubilize phosphate and micronutrients that could be made available to provide plant growth. They also concluded that the improvement of plant nutritional level might be directly related to a general beneficial growth effect of the root system following *T. harzianum* inoculation. The results of present study was in line of earlier studies which indicated that *T. harzianum* had a positive effect on citrus transplant growth.

Table 5. Effect of compost fortified with *T. harzianm* and Top.Zn on the nutritional status of two citrus seedlings infected with *F. solani* and *M. phaseolina*

Nutritional status / Treatments	Untreated	Soil infested with								
			F. solani				*M. phaseolina*			
		T. harz	Infes.	*T. harz.*	Top.Zn	T. harz	Infes.	*T. harz.*	Top.Zn	Top.Zn + *T. harz*
Orange										
Macronutrients [%]										
N	* 1.77e	2.30d	1.77e	1.70g	3.35b	3.47a	1.73f	1.46h	2.50c	3.46a
P	0.214b	0.215b	0.176c	0.158e	0.236a	0.238a	0.164d	0.112f	0.215b	0.238a
K	1.72ab	1.78ab	1.52ab	1.78ab	1.88ab	2.32a	1.40b	1.03b	1.78ab	2.00ab
Micronutrients [µ/ g^{-1}]										
Fe	60.0e	73.6bcd	70.0cd	69.3d	75.0b	88.0a	69.7cd	73.0bcd	74.3bc	75.3ab
Zn	41.7ab	43.0ab	39.0b	41.0ab	44.0ab	45.3a	39.0b	39.0b	43.3ab	45.0a
Mn	27.7c	39.0bc	27.3c	36.3c	41.0ab	43.0a	27.0e	37.8c	40.3ab	41.0ab
Cu	5.7f	7.3b	6.9c	6.1e	7.5ab	7.6ab	6.7d	7.0c	6.7d	7.6a

Mandarin										
Macronutrients [%]										
N	1.54g	2.13d	1.93e	1.96e	2.43c	2.91a	1.81f	1.94e	2.09d	2.69b
P	1.00e	0.175b	0.164bc	0.139d	0.213a	0.215a	0.160c	0.165bc	0.206a	0.213a
K	1.32b	2.32a	1.40b	1.52ab	1.88ab	2.00ab	1.33b	1.72ab	1.78ab	1.78ab
Micronutrients [µ/ g^{-1}]										
Fe	69.0c	87.70a	63.0d	70.7bc	73.0bc	72.0bc	69.7bc	70.0bc	72.7bc	73.3b
Zn	35.7ab	45.3a	37.0cd	41.1abc	39.0bcd	43.0ab	39.0bcd	40.7abc	41.7abc	43.7ab
Mn	28.0d	39.0c	36.7c	37.3c	40.7ab	42.3a	37.6c	38.7bc	40.7ab	41.3ab
Cu	5.8c	7.1ab	6.9ab	6.6b	7.3ab	7.7a	6.8ab	7.0ab	7.2ab	7.4ab

*Means values followed by the same letter within the treatments are not significantly different ($P < 0.05$) according to the Duncan's multiple range tests.

3. Biological activity populations

Tables 6 and 7 show the effect of compost fortified with *T. harzianum* and Top.Zn on the microflora of the two citrus seedlings. It is clear that the total fungal and total bacterial counts in the rhizosphere of both orange and mandarin were increased progressively as the plant grew up reaching their maximum at the last count which was taken after 90 days. This increase may be attributed to the increase around and richness of root exudates and sloughs. Rhizosphere microorganisms utilize compounds and materials released from the roots and provide microorganisms with nutrients, consequently, the rhizosphere supports large active microbial populations (Akhtar and Siddiqui 2008)

Table 6. Effect of compost fortified with *T. harzianm* and Top.Zn on the total fungal counts of two citrus seedlings infected with *F. solani* and *M. phaseolina*.
(cfu x10^3/g soil)

Nutritional status / Treatments	Untreated	Soil infested with: *T. harz*	*F. solani*: Infes.	*F. solani*: *T. harz.*	*F. solani*: Top.Zn	*F. solani*: Top.Zn + *T. harz.*	*M. phaseolina*: Infes.	*M. phaseolina*: *T. harz.*	*M. phaseolina*: Top.Zn	*M. phaseolina*: Top.Zn + *T. harz.*
Orange										
After 30 days	3.0	182.6	21.9	34.7	12.4	11.1	28.5	82.6	9.2	6.4
After 60 days	14.2	1641.9	28.7	81.9	7.3	104.4	19.1	181.0	23.8	27.9
After 90 days	1670.2	3348.7	138.0	417.2	81.9	89.9	2560.7	4540.0	144.9	117.0
Mean	56.2	1724.4	62.9	177.9	33.9	68.5	869.4	1628.7	59.3	50.4
Mandarin										
After 30 days	0.6	47.3	7.8	73.7	0.8	26.9	1.1	67.8	2.3	33.0
After 60 days	23.9	80.9	34.4	48.1	9.1	27.8	32.0	425.3	13.9	38.4
After 90 days	120.5	406.5	160.8	254.8	39.1	82.8	261.1	2693.9	85.42	43.0
Mean	48.3	178.2	67.7	125.6	16.3	45.8	98.1	1062.3	33.9	38.1

a-Total fungal count

Inoculation of *T. harzianum* to soil alone increased the total fungal community in the rhizosphere of orange from 56.2 to 1724.4 cfu/g (30.7

times) and from 40.3 to 178.2 cfu/g (4.42 times) in mandarin. Fungal population in the rhizosphere of infested soil with *F. solani* or *M. phaseolina* was stimulated than that of the comparable healthy ones. As, inoculation of orange and mandarin citrus plant with *F. solani* resulted in an 11.92% and 40.16% increase in fungal count, respectively. The same trend was also observed when soil inoculated with *M. phaseolina.* Such infection would lead to the damage of the root and hence resulting in increased activity of saprophytic microorganisms.

Treatment with compost fortified with *T. harzianum* to infested soil with *F. solani* and *M. Phaseolina* increased the fungal population density by 3.34 and 28.98 folds respectively in orange and by 2.60 and 21.99 times respectively in mandarin compared with non treated control. As reported by Hoitink *et al.* (1991) that compost treatment cause a slow release of nutrients which supports beneficial activity of microflora.

Data in Table 6 also revealed that soil treated with Top.Zn alon decreased the total fungal count in the rhizosphere of orange from 56.2 to 33.9 cfu/g (39.18% decrease) and from 48.3 to 16.3 (66.25% decrease) in the rhizosphere of mandarin. It is proved that Top.Zn may have been fungicide effect toward fungi in soil. The same trend was also observed when Top.Zn was treated in combination with *T. harzianum*. Similar inhibitory effects of fungicides were recorded by Domsch (1960) ; Sahab *et al.* (1985).

b-Total bacterial count

It is clear from Table 7 that treatment of orange and mandarin seedlings with compost fortified with *T. harzianum* alone decreased the total bacterial counts from 488.1 to 58.9 cfu/g (87.93 % decrease) and from 549.6 to 124.5 cfu/g (77.35 % decrease) respectively.

Table 7. Effect of compost fortified with *T. harzianm* and Top.Zn on the total bacteria of two citrus seedlings infected with *F. solani* and *M. phaseolina* .
(cfu x10^5/g soil)

Nutritional status / Treatments	Untreated	Soil infested with								
		T. harz	*F. solani*				*M. phaseolinea*			
			Infes.	*T. harz.*	Top.Zn	Top.Zn + *T. harz*	Infes.	*T. harz.*	Top.Zn	Top.Zn + *T. harz*
Orange										
After 30 days	8.0	43.9	73.56	90.4	73.7	93.7	18.2	138.6	35.14	31.6
After 60 days	17.9	31.9	166.1	61.9	44.7	101.4	32.1	37.9	68.1	55.9
After 90 days	1438.3	2.5	1003.4	2031.6	3039.8	23.9	2732.9	178.6	2207.2	22.13
Mean	488.1	26.1	635.0	727.9	1052.7	73.0	927.7	118.4	770.1	102.9
Mandarin										
After 30 days	37.6	11.7	32.7	15.0	14.7	11.2	13.8	29.9	36.9	13.3
After 60 days	37.6	26.3	21.8	13.6	28.5	17.7	23.7	32.2	199.6	15.9
After 90 days	1573.6	335.4	773.8	583.9	332.0	641.8	386.8	474.1	458.3	150.5
Mean	549.6	124.5	276.1	204.2	125.1	223.6	141.3	178.7	231.6	59.9

In this respect many investigators noted that compost treatment highly reduced population density of some fungi and bacteria. In addition, rhizobacteria which produce antibiotic that suppress deleterious microbes (El-Mohammedy and Ahmed 2009 ; Ziedan, 2000). On the other hand, the average number of bacteria was much higher in the rhizosphere of orange soil infested with *F. solani* and *M. phaseolina* than in non-infested soil (control). While opposite trend was observed in the rhizosphere of mandarin, as the average number of total bacterial count were decreased from 549.6 to 226.1 cfu/g (49.76% decrease) and from 549.6 to 141.3 cfu/g (74.29% decrease) in soil infested with *F. solani* and *M. phaseolina,* respectively.

In infested soil with *F. solani* and *M. phaseolina* , the treatment with Top.Zn and compost fortified with *T. harzianum* decreased the average number of total bacterial counts in the rhizosphere of orange by 85.04 and 78.92%, respectively and by 59.32 and 92.74 %, respectively in the rhizosphere of mandarin.

The obtained results throughout the present work highlighted the efficiency of compost as appropriate carriers for *T. harzianum* and Top.Zn used as biological control of citrus root-rot disease.

REFERENCES

1. Akhtar M. S., Siddiqui Z. A. 2008. Biocontrol of root-rot disease complex of chickpea by *Glomus intraradices, Rhizobium* sp. and *Pseudomonas straita*. Crop protection 27: 410-417.
2. Allen O. N. 1961. Experiments in soil bacteriology. Burgess Pub. Co. USA. 147 pp.
3. Altomare C., Norvell W. A., Bjorkman T., Harman G. E. 1999. Solubilization of phosphates and micronutrients by the plant growth promoting and biocontrol fungus *T. harzianum* Rifai,1295-22. Applied Environ. 65: 2926-2933.
4. A.O.A.C. 1990. Official Methods of Analysis Association of Official Analytical Chemists. 15th Ed. Inc. Wash. D.C.
5. Barnett H.L., Hunter B. B. 1996. Illustrated genera of imperfect fungi. Burgess Pub. Co. Minnesota, 241 pp.
6. Booth C. 1971. The genus *Fusarium.* Commonwealth Mycological, Institute, Kew, Surrey, England, 273 pp.
7. Buent J. S., Rovira A. O. 1955**.** Microbiological studies of some subantarctic soils. J. Soil Sci. 6: 119.
8. Catara A., Polizzi G. 1999. Dry root rot of citrus symptoms, causing and susceptibility of rootstock. Rivisto di fruticolture 6: 38-41.
9. Coombs P. O., Hall S. P., Schlock, J. M. O. 1987. Techniques in bioproductivity and photosynthesis. Pergamon Press, Oxford, 159.
10. Cottenie A., Verloo M., Velghe G., Comerlynk R. 1982. Chemical analysis of plant and soil. Ghent, Belgium, Laboratory of Analytical and Agro-chemistry State University.
11. De Couster T. J. H., Hoitink A. J. 1999. Using compost to control plant diseases. Bio Cycle Magazine, June: 61.
12. Domsch K. H. 1960. The efficiencies of soil fungicides. IV. Spectrum changes in the soil flora. Z. Pjkrankh. 67: 129.
13. Dubeikovsky A. N., Mordukhova E. A., Kochetkov V. V., Polikarpova F. Y., Boronin A. M. 1993. Growth promotion of blackcurrant softwood cuttings by recombinant strain *Pseudomonas fluorescens* BSP53a synthesizing an increased amount of indol-3-acetic acid. Soil Biology and Biochemistry 25: 1277-1281.
14. Duncan D. B. 1955. Multiple range and multiple F Test. Biometrics 11: 1-42.
15. Elad Y. 2000. Biological control of foliar pathogens by means of *Trichoderma harzianum* and potential modes of action. Crop Protection 19: 709-714.
16. El-Mohamedy R. S., Ahmed M. A. 2009. Effect of biofertilizers and humic acid on control of dry root-rot disease and improvement yield quality of mandarin. Research J. Agric. And Biol. Sci. 5: 127-139.
17. Gilman C. J. 1957. A manual of soil fungi. 2nd ed. Iowa State College Press, 45o pp.
18. Haggag W. M., Saber M.S. 2000. Evaluation of three composts as multi-strain carriers for biofertilizer and biocontrol agents against *Fusarium* wilt of some legume plant. Arab J. Biotech. 13: 133-144.
19. Harman G. E., Latorre B., Agosin E., Martin R.S., Riegel R. S., Nielson P. A., Tronsmo A., Pearson R. C. 1996. Biological control and integrated control of Botrytis Bunch rot of grape using *Trichoderma* spp. Biological control 7: 259-266.
20. Healther M. D., Alexandra G. S., Richard P.D. 2006. Compost and manure mediated impacts on soilborne pathogens and soil quality. Soil Science Society of American J. 70: 347-358.
21. Hoitink H. A. J., Inbar Y., Boehm M. J. 1991. Status of compost-amended potting mixes naturally suppressive to soilborne diseases of floricultural crops. Plant Disease 75: 869-873.
22. Ibrahim M. D. 2005. Delivery system of *Trichoderma* inoculants for control of *Rhizoctonia* disease in *Brassica rapa*. Ph.D. Thesis, UPM, Malysia.
23. Jones Jr. J. B., Wolf B., Mills H. A. 1991. Plant analysis Handbook. Micro-Macro Publishing. Inc., Georgia, USA. Chapter 7. pp.45-88.
24. Kore S. S., Mane A. V. 1992. A dry root disease of Kagilime (*Citrus aurantifolia*) seedlings caused by *Fusarium solani*. Hournk Maha Aric. India 17: 276-278.
25. Kung'u J. N., Sief A.A., Gatumbi R. W. 2002. *Macrophomina* root rot of citrus in Kenya, E. Afr. Agric. For. J. 68: 1
26. Louw H. A., Weblely D. W. 1959. The bacteriology of root region of the oat plant grown under controlled pot culture conditions. J. Appl. Bacteriol. 22: 216
27. Muhammed S., Amusa N. A. 2003. *In-vitro* inhibition of growth of some seedling blight inducing pathogens by compost inhabiting microbes. African J. of Biotechnology 2: 161-

164.
28. Nelson P. E., Toussoum T. A., Marasas W.F.O 1993. *Fusarium* spp. An illustrated manual for identification. Philadelphia(PA): Pennsylvania Univ. P. 189.
29. Ousley M. A., Lynch J. M., Whips J. M. 1993. Effect of *Trichoderma* on plant growth ;a balance between inhibition and growth promotion. Microbial. Ecology 26: 277-285.
30. Ozbay U., Newman, S. 2004. Effect of *Trichoderma harzianum* strains to colonize tomato roots and improve transplant growth. Pakistan J. Biol. Sci. 7: 253-257.
31. Peterburgski A.V. 1968. Handbook of Agronomic Chemistry. Kolop Publishing House, Moscow, Russia.
32. Rensburg J. C., Labuschagne N., Nemic S. 2001. Occurrence of *Fusarium*-produced naphthazarins in citrus trees sensitivity of relation to citrus blight. Plant Pathology 50: 258-265.
33. Rivera M. C., Wright E. R., Lopez M. V., Garda D., Barraque M. Y. 2004. Promotion of growth and control of damping – off (*R. solani*) of greenhouse tomatoes amended with vermicompost. Phyton 04: 229-235.
34. Sahab A. F., Osman A. R., Soleman N. K., Mikhail M. S. 1985. Studies on root-rot of lupin in Egypt and its control. Egypt. J. Phytopath. 17(1): 23-35.
35. Scheuerell S. J., Mahaffe W.F. 2004. Compost tea as a container medium drench for suppressing seedling damping-off caused by *P. ultimum*. Phytopathology 94: 1156-1163.
36. Siddiqui Y., Meon S., Ismail R. Rahmani M., Ali A. 2008. Bio-efficiency of compost extracts on the wet rot incidence , morphological and physiological growth of okra (*Abelmoschus esculentus*) Sci. Horticulturae 117: 9-14.
37. Strauss J., Labuschagen N. 1999. Pathogenicity of *Fusarium solani* isolates on citrus roots and evaluation of different inoculum types. Toage Paste Plant Watenskap 9: 48-52.
38. Sylvia E. W. 2004. The effect of compost extract on the yield of strawberries and severity of *Botrytis cinerea*. J. of Sustainable Agriculture 25: 1-8
39. Verma K. S., Nartey S., Singh N. 1999. Occurrence and control of dry root rot of citrus seedlings. Plant Disease Research 14: 31-34.
40. Windham M. T., Elad Y., Baker R. 1986. A mechanism for increased plant growth induced by *Trichoderma* spp. Phytopathol. 76: 518-521.
41. Ziedan H. E. 2000. Soil treatment biofertilizers for controlling peanut rot and pod rot disease in Nobaria province. Egypt J. Phytopathol. 28: 17-26.
42. Yedidia I., Srivastva A. K., Kapulnik Y., Chet I. 2001. Effect of *T. harzianum* on microelement concentrations and increased growth of cucumber plants. Plant and Soil 235: 235-242.

2/1/2010