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Biological Soil Treatment to Control *Fusarium solani* and *Tylenchulus semipenetrans* on Sour Orange Seedlings Under Greenhouse Conditions

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Abstract : Dry root rot and slow decline diseases of citrus caused by *Fusarium solani* and *Tylenchulus semipenetrans*, respectively, are serious diseases attacking many groves in Egypt. We evaluated the efficiency of soil amended with bio-agents and compost alone or in combination to control both diseases simultaneously on sour orange (*Citrus aurantum*) seedlings under greenhouse conditions. All tested bioagents reduced *T. semipenetrans* population densities and the linear growth of *F. solani*. Complete inhibition of the linear growth was obtained with *Trichoderma viride*, *T. harzianum* isolate no 3 and *Bacillus subtilis* isolate no 4. The compost with each of *Bacillus subtilis*, *Trichoderma harzianum* or *T. viride* could reduce the rate of nematode build-up to 0.35, 0.38, and 0.41; respectively. The most effective treatment against *F. solani* was compost + mixture of *T. harzianum* + *T. viride* which reduced disease incidence and severity by 87.5%. The highest reduction in total count of *F. solani* was obtained with compost + mixture of *T. harzianum* + *T. viride* which reduced total count by 82.1%. Treatment with compost alone could increase ($P \leq 0.05$) fresh weight of sour orange roots over that treated with *F. solani* and/or *T. semipenetrans.* Other treatments were less effective. The highest increase in enzyme activities was obtained with combined treatments of compost and *T. harzianum*, *T. viride*, *B. subtilis* (or *T. harzianum* + *T. viride*) which increased the peroxidase, polyphenol oxidase and chitinase activities 300, 72.2 and 109.9%, respectively.
Key words: Keywords: Biocontrol, compost, *Fusarium solani*, greenhouse, sour orange, *Tylenchulus semipenetrans*.

Introduction

Dry root rot disease of citrus caused by *Fusarium solani* (Mart.) is one of the most serious diseases attacking citrus trees especially in newly reclaimed lands in Egypt, it has been estimated to adversely affect 8.9% of lime trees and caused 39.6% loss in fruit yield 1. This disease was reported to attack all citrus varieties in Egypt[1,2.] Likewise, slow decline disease of citrus caused by the citrus nematode *Tylenchulus semipenetrans* (Cobb) is another important disease worldwide where nematode population levels may affect fruit yield differently under various conditions [3-,8.]

Both pathogens can infect and thrive on sour orange; the most common rootstock in Egypt so far.

Control of the two diseases depends mainly on fungicides and nematicides application[9,5.] Such chemicals are not always desirable due to potential hazards to human beings and the environment. Biological

control agents and soil amendments individually or in combination are among the recent recommended alternatives for controlling the nematodes and soil borne pathogens.[10,11.]

Using agricultural and domestic food wastes or some grains as substrates for *T. harzianum* growth, formulation and/or direct delivery in soil for controlling phytonematodes and soil borne pathogens on economically important crops were recorded.[12-17.]

In Egypt, such alternative control methods are needed for managing these pathogens. The application of biological control using antagonistic microorganisms *i.e*., *Trichoderma harzianum, T. viride* and *Bacillus subtilis*, proved to be successful for controlling various phytonematodes and soil-borne plant diseases on different crops[18-24]. Many soil-borne pathogens can be reduced by application of composts made of different raw materials[25]. Currently it is believed that a combination of antagonistic microorganisms with mature compost may be more efficient in inhibiting diseases than using single antagonistic microbial strain or compost alone[25-27].

The purpose of this research is to evaluate the efficiency of soil amended with bio-agents and compost alone or in combination to control the fusarium dry root rot and slow decline diseases under greenhouse conditions. Activities of three enzymes, that may be involved in inducing resistance of plants to the attacking pathogens, were measured.

Materials and Methods

***Source of pathogenic fungus and bio-agents*:** Pathogenic isolate of *F. solani* the causal agent of dry root rot disease of citrus plants and antagonistic strains of *Trichoderma harzianum, T. viride* and *B. subtilis* were obtained from Plant Pathology Dept. at the National Research Centre.

***Source of plant compost*:** Chemical analysis and properties of **t**he plant compost obtained from El-Nile Company, Egypt were as follows: total nitrogen 1.53%, total phosphorus 0.29%, total potassium 1.13%, total iron (1368 ppm), total zinc (70 ppm), total copper (12 ppm), total organic matter (31%), organic carbon 33.4%, carbon / nitrogen ratio 12:1, pH (1:100) 8.1, EC 1:100, and humidity (8.54%).

Laboratory experiment:

Evaluation of antagonistic effect of some rhizospheric fungi on the linear growth of F. solani:

Four antagonistic isolates of *T. harzianum, T. viride* and *B. subtilis* were tested to study their inhibitory effect against *F. solani in vitro*. Each of the obtained fungal antagonists, and pathogenic fungus were grown on Potato Dextrose Agar (PDA) medium for 7 days at 25 ± 2°C. Disk of individual antagonistic fungi and disk of *F. solani* were placed on opposite sides of Petri plates containing PDA medium [28]. Inoculated plates were incubated for 7 days at 25 ± 2°C. Five plates for each particular treatment/isolate were used as replicates. The plates were then examined and growth area of *F. solani* was measured. The reduction percent in linear growth of *F. solani* was calculated.

Evaluation of antagonistic effect of B. subtilis on the linear growth of F. solani :

Four isolates of B. subtilis were tested to study their inhibitory effect against *F. solani in vitro*. Antagonistic isolates were grown individually on nutrient agar medium (NA) for 48 h at 30 ± 2°C. Using dual culture plate assay, 6-mm-diameter disk of *F. solani* was placed near the edge of Petri plate containing PDA medium. Loop of bacterial isolate was placed as individual streak in the opposite of *F. solani* [29]. Inoculated plates were incubated for 7 days at 25 ± 2°C. Five plates for each particular treatment were used as replicates. The plates were then examined and linear growth of *F. solani* was measured and the reduction percent in linear growth was calculated.

Greenhouse experiment:

1) Evaluating the efficiency of soil amended with bio-agents and compost alone or in combination on dry rot and slow decline diseases of citrus seedlings.

Inoculum Preparation of biocontrol agents: T. harzianum and T. viride were grown on PDA medium at 25 ± 1°C for 10 days; afterwards the mycelium with the spores was scraped from Petri plate and mixed with sterilized distilled water (20 ml/plate) in a blender. The suspension was adjusted by hemocytometer slide to 3×10^6 colony forming units (cfu)/ml as described by[30]. Also, one loop of *B. subtilis* isolate was inoculated into Nutrient Broth medium (g/l): peptone 5 g, beef extract 3 g, sodium chloride 5 g, glucose 20 g, pH 7; and incubated on a shaker incubator (125 rpm) at 28 ± 1°C. Antagonistic bacterial cells were then harvested after 48 hours of growth in culture medium by centrifugation at 6,000 rpm for 15 min and re-suspended in a phosphate buffer (0.01 M, pH 7.0). The concentration was adjusted by plate count technique to an approximately 3×10^7 cfu/ ml as mentioned by [30].

Soil infestation with *F. solani*

Sandy-loam soil was autoclaved at 120°C for 1 h. Plastic pots (30 cm diameter, 5 kg soil) containing sterilized sandy-loam soil were artificially infested with the inoculum of *F. solani* at the rate of 50 ml (10^6 cfu/ ml) / kg soil [31]. One 2-year-old healthy seedling of the most widespread commercial rootstocks in Egypt, *i.e.*, Sour orange (*Citrus aurantum* L.) was transplanted to each pot. The seedlings were obtained from Citrus Research Division, Agriculture Reseach Center, Giza, Egypt.

Preparation and inoculation of Tylenchulus semipenetrans

Soil and root subsamples were obtained from 'Baladi' mandarin (*Citrus reticulata* Blanco) orchard [32] with a hand trowel (ca 6 cm diam X 30 cm deep) beneath the tree canopy at 1.25 m from the trunk and mixed. The subsamples were composited into a single sample of about 1000 cm^3 representing a random tree. Samples were bagged, labeled and taken to the laboratory for nematode extraction and count. Ninety samples were thoroughly mixed from which ten 250-cm^3 soil samples were processed for extraction of citrus nematode juveniles (J2) and males while the fibrous roots were washed from the soil to recover females of the citrus nematode[33]. Key reference of [34] was consulted to identify *T. semipenetrans* via examining several mounted adult females in glycerin. All counted live *T. semipenetrans* J2 and males were retuned back to the bulk soil which was caliberated so that each 1 kg soil contained 3000 *T. semipenetrans* J2 and males. Five days after the addition of *F. solani*, 1 kg soil with 3000 *T. semipenetrans* individuals was added per pot.

Preparation of bio-compost

Inocula of antagonistic fungi isolates, *i.e. T. harzianum* or *T. viride* (10^{12} cfu/ml) in addition to the antagonistic bacteria, *i.e. B. subtilis*, (10^{16} cfu/ ml) were prepared as mentioned before. Plant compost was autoclaved at 120°C for 60 min. Fungal and bacterial suspensions were added individually to sterilized plant compost at the rate of 2:1 (Plant compost: suspension, W:V), then mixed thoroughly to ensure equal distribution of microorganism suspension through the plant compost. The prepared mixture was placed on paper sheet and left for air dry 4-6 hrs at room temperature (22-25 °C) in laminar flow under sterilized conditions.

Soil treatment with compost and/or bioagents: Soil infested with *F. solani* and *T. semipenetrans* was treated with bio-agent fungi or bacteria at the rate of 50 ml (10^6 cfu/ ml)/ kg soil of each antagonistic fungi or bacteria. Plant compost or bio-compost (compost + bio agents) was added to soil infested with *F. solani* and *T. semipenetrans* at the rate of 50 g/ kg soil. The compost and/or bioagent (s) were added 5 days after nematode inoculation.

Treatments

The treatments can be summarized as follows: 1) Non infested soil, 2) soil infested with *F. solani*, 3) soil infested with *T. semipenetrans*, 4) Soil infested with *F. solani* (FS) + *T. semipenetrans*(TS), 5) soil infested with (FS + TS) + plant compost (PC), 6) soil infested with (FS + TS) + *T. harzianum*, 7) soil infested with (FS + TS) + *T. viride*, 8) soil infested with (FS + TS) + *B. subtilis*, 9) soil infested with (FS + TS) + PC + *T. harzianum*, 10) soil infested with (FS + TS) + PC + *T. viride*, 11) soil infested with (FS + TS) + PC + *B. subtilis*, and 12) soil infested with (FS + TS) + PC+ *T. harzianum*+ *T. viride*. Ten replicates/seedlings were used for each treatment.

Disease assessment:

Ninety days after treating sour orange pots with compost and/or bioagents in the greenhouse, the development of disease severity of Fusarium root rot disease on seedlings of each treatment was estimated on 0-4 scale (0 = healthy plant and 4 = died plant) according to[35]. Percentages of disease infection and severity of each treatment were calculated.

Count of *Fusarium solani* in rhizosphere soil

Plate count technique using Peptone-pentachloronitrobenzene (PCNB) agar medium and PDA medium supplement with 250 ppm chloromycetin was used according to [6] to determine total counts of *Fusarium solani* in rhizosphere soil of each treatment. Five plates were used as replicates for each treatment. Total count of fungus was expressed as cell forming units per gram dry soil.

Assessing parameters of *Tylenchulus semipenetrans* population and plant growth:

Ninety days after treating sour orange seedlings with compost and/or bioagents in the greenhouse, the plants were carefully removed from soil, the shoot systems of the tested plants were cut off and the roots gently washed from the soil. *Tylenchulus semipenetrans* counts as number of J2 and males in 250 g soil and number of females and eggs in 5 g roots per plant were recorded. The rate of *T. semipenetrans* build-up and the nematicidal activity, if any, of each treatment on *T. semipenetrans* population were reported. Also, after removing plants from soil, lengths and fresh weights of the shoots and lengths and fresh weights of roots were recorded.

2) Evaluating the efficiency of soil amended with bio-agents and compost alone or in combination on enzyme activities of sour orange seedlings.

Extraction of enzymes: Sour orange root samples (2g/pot) were taken soon after plant removal and ground in a mortar in the presence of purified sand plus 4 ml of 0.1 M sodium phosphate buffer (pH 7.1) 37. The homogenate was strained through four layers of cheesecloth then the filtrates were centrifuged at 3000 rpm for 20 min. at 6°C. The obtained supernatant fluids (crude enzyme extracts) were used for assaying activities of peroxidase, polyphenol-oxidase (PPO) and chitinase enzymes at 425, 420 and 540nm, respectively using Spectrophotometer (Spectronic 20-D). Enzyme extract was replaced by distilled water in control blank cuvette.

Peroxidase assay:

Peroxidase activity was determined according to the method described by [38]. The cuvette contained 0.5 ml. 0.1 M potassium phosphate buffer at pH 7.0 + 0.3 ml of enzyme extract + 0.3 ml 0.05 M pyrogallol + 0.1 ml 1.0% H_2O_2 and distilled water to bring cuvette contents to 3.0 ml. The reaction mixture was incubated at 25°C for 15 min. Peroxidase activity was expressed as the increase in absorbance at 425 nm/g fresh weigh.

Polyphenol-oxidase assay: The polyphenol-oxidase activity was determined according to the method described by [39]. The reaction mixture contained 0.2 ml enzyme extract, 1.0 ml of 0.2 M sodium phosphate buffer at pH 7.0 and 1.0 ml 10^{-3} M catechol and complete with distilled water up to 6.0 ml. The reaction mixture was incubated for 30 min at 30°C. Polyphenol-oxidase activity was expressed as the increase in absorbance at 420 nm/g fresh weigh.

Chitinase assay: Chitinase activity was spectrophotometrically measured (at optical density 540 nm/g fresh wight/60 min) according to the method of [40].

Statistical analysis: Data were subjected to analysis of variance (ANOVA) and averages of shoot and root weights and lengths as well as numbers of each nematode developmental stage were compared using Duncan's New Multiple Range Test. Nematode counts were log-transformed before ANOVA since *T. semipenetranshas* showed contagious distribution.[41].

Results

Evaluation of antagonistic effect of some bioagents on the linear growth of *Fusarium solani*: Four antagonistic isolates of *Trichoderma harzianum*, *T. viride* and *B. subtilis* were tested to study their inhibitory effect against *F. solani in vitro*. Results in Table (1) indicate that all tested bioagents significantly reduced the linear growth of *F. solani*. Complete inhibition of linear growth was obtained with *T. harzianum* (isolate no. 3), *T. viride* and *B. subtilis* (isolate no. 4). The highest reduction was obtained with *T. viride* (isolate numbers 2 and 3) which reduced the linear growth by 78.8 %. Other isolates showed moderate effect.

Greenhouse experiment: This experiment was carried out to evaluate the efficacy of bio-agents and compost alone or in combination for controlling Fusarium dry root rot disease and citrus slow decline under greenhouse conditions.

Effect on root rot disease of sour orange: Results in Table (2) revealed that the most effective treatment was compost + mixture of *T. harzianum* + *T. viride* which reduced disease incidence and severity by 87.5%. The highest reduction was obtained with compost + *T. harzianum* or *T. viride* which reduced disease incidence and severity by 75%. Other treatments were less effective.

Effect of bioagents and/or compost on total count of *Fusarium solani* in sour orange rhizospheric soil: Results in Table (3) indicated that all treatments significantly reduced the total count of *F. solani* in rhizospheric soil of sour orange seedlings. The highest reduction in total count of *F. solani* was obtained with compost + mixture of *T. harzianum* + *T. viride* which reduced total count by 82.1. This was followed by infested soil treated with compost + *T. harzianum* or *T. viride* in a descending order of efficiency. Other treatments were less effective.

Effect of bioagents and/or compost on parameters of *T. semipenetrans* populations and plant growth: Nematode development and reproduction were reduced by all treatments (Table 4). This was quite clear since the rate of nematode build up was < 1; that is, nematode final population is less than nematode initial population in all treatments. The data suggest that the treatments delayed nematode development or did not allow the nematode to complete its life cycle by the end of the experiment. The greatest reduction in total numbers of *T. semipenetrans* was caused in the treatment of compost + *B. subtilis* + *F. solani*, followed by compost + *T. harzianum* + *F. solani*, compost + *T. viride* + *F. solani*, and *B. subtilis* + *F. solani* in a descending order. Other treatments were less effective. Treatment with compost alone could increase ($P \leq 0.05$) fresh weight of sour orange roots over that treated with *F. solani* and/or *T. semipenetrans* (Table 5). Effect of bioagents and/or compost on other plant growth parameters were only numerically different.

Impact of soil amended with bio-agents and compost alone or in combination on enzyme activities of sour orange seedlings: Results in Table (6) revealed that all tested treatments significantly increased the tested enzyme activities. The most effective treatments were compost combined with *T. harzianum* and/or *T. viride*, or combined with *B. subtilis* which increased the peroxidase, polyphenol oxidase and chitinase activities. Single treatments were less effective.

Table 1. Effect of bio-agents on linear growth of *Fusarium solani* (n = 5)*.

Bio agent	Isolate number	*Fusarium solani*	
		Linear growth	Reduction %
Trichoderma harzianum	1	34 c	62.4
	2	27 d	70
	3	0.0 f	100
	4	15 e	83.3
Trichoderma viride	1	40 b	55.2
	2	19 e	78.8
	3	19.0 e	78.8
	4	0.0 f	100
Bacillus subtilis	1	0.0 f	100
	2	46 b	48.8
	3	32 cd	64.4
	4	0.0 f	100
Control	——	90.0 a	0

*Means in a column followed by the same letter are not significantly ($P \leq 0.05$) different according to Duncan's New Multiple Range Test.

Discussion

Fusarium root rot disease caused by *F. solani* and citrus slow decline incited by *T. semipenetrans* are two of the most serious diseases attacking citrus trees especially those cultivated in newly reclaimed lands of Egyptian desert[1,5,32]. All tested treatments (Tables 1-6) were effective and could serve as environmentally friendly products that can replace hazardous chemical fungicides and nematicides.

The application of biological control using antagonistic microorganisms *i.e*., *Trichoderma harzianum, T.viride* and *Bacillus subtilis*, proved to be successful for controlling various soil-borne plant diseases in many countries[11,21-24]. In the present study, all tested bioagents significantly reduced the linear growth of *F. solani*. Complete inhibition of linear growth was obtained with *T. harzianum* (isolate no 3), *T. viride* and *B. subtilis* (isolate no 4). The highest reduction was obtained with *T. viride* (isolate numbers 2 and 3) which reduced the linear growth by 78.8%. Moreover, under greenhouse experiment results revealed that the most effective treatment was compost + mixture of *T. harzianum* + *T. viride* which reduced disease incidence and severity by 87.5% (Table 2).

Table 2. Effect of biological soil treatments on root rot complex of sour orange caused by *F. solani* and *T. semipenetrans* under greenhouse conditions (n = 10)*.

Soil treatment	**Root rot incidence**			
	Disease infection	**Reduction %**	**Disease severity**	**Reduction %**
Fusarium solani (FS)	60 b	——	40 a	——
Tylenchulus semipenetrans (TS)	——	——	——	——
FS + TS	80 a	0.0	40 a	0.0
Trichoderma harzianum (Th) + (FS + TS)	40 c	50	10 d	75.0
Trichoderma viride (Tv) + (FS + TS)	40 c	50	10 d	75.0
Bacillus subtilis (Bs) + (FS + TS)	40 c	50	30 b	25
Compost +Th + (FS + TS)	20 d	75	10 d	75.0
Compost +Tv + (FS + TS)	20 d	75	10 d	75.0
Compost +Bs + (FS + TS)	20 d	75	20 c	50
Compost +Tv + Th + (FS + TS)	10 e	87.5	5 de	87.5
Compost + (FS + TS)	40 c	50	30 b	25
Control	0.0 f	0.0	0.0 e	0.0

*Means in a column followed by the same letter are not significantly ($P \leq 0.05$) different according to Duncan's New Multiple Range Test.

Table 3. Effect of biological soil treatments on *F.* solani population density in sour orange rhizosphere soil inoculated with *F. solani* and *T. semipenetrans* under greenhouse conditions*.

Soil treatment	Average propagules of *F. solani* (cfu X 10^5/ g dry soil)	
	Total count	Reduction %
F. solani (FS)	47.8 a	
T. semipenetrans (TS)	——	——
FS + TS	52.4 a	0.0
T. harzianum (Th) + FS + TS	20.2 d	61.5
T. viride (Tv) + (FS + TS)	23.2 d	55.5
Bacillus subtilis (Bs) + (FS + TS)	30.4 c	42.0
Compost +Th +(FS + TS)	12.8 ef	75.6
Compost +Tv + (FS + TS)	14.6 e	72.1
Compost +Bs + (FS + TS)	18.8 d	64.1
Compost +Tv + Th +(FS + TS)	9.4 f	82.1
Compost +(FS + TS)	36.8 b	29.8

*Means in a column followed by the same letter are not significantly ($P \leq 0.05$) different according to Duncan's New Multiple Range Test (n = 10).

Table (4): Effect of bio-control agents on *T. semipenetrans* population parameters on sour orange under greenhouse conditions after 3 months of treatments*.

Soil treatment	Nematode parameters				Final population		*Rate of nematode build up*
	J_2 + males in 250 g soil		Roots: Females and Eggs (total) in 5 g roots				
	Count	Reduction (%)	Count	Reduction (%)	Count	Efficacy (%)	
F. solani (FS)	——	——	——	——	——	——	——
T. semipenetrans (TS)	481 a(3)	----	3555 a	----	4036	----	*1.35*
FS + TS	366 b	23.9	2363 a	33.5	2729	32.4	*0.91*
T.h + TS + FS	340 b	29.3	1863 a	47.6	2203	45.4	*0.73*
T.v TS + FS	400 ab	16.8	1994 a	43.9	2394	40.7	*0.80*
B.s + TS + FS	389 ab	19.1	950 b	73.3	1339	66.8	*0.45*
Compost +T.v + TS + FS	351 b	27.0	880 b	75.2	1231	69.5	*0.41*
Compost +T.h + TS + FS	283 b	41.2	843 b	76.3	1126	72.1	*0.38*
Compost + B.s + TS + FS	330 b	17.9	730 b	79.5	1060	73.7	*0.35*
Compost +Tv + Th + (FS + TS)	*283 b*	*41.2*	*730 b*	*79.5*	*1060*	*73.7*	0.35

*Nematode-initial population = 3000 J_2 + males. Means in a column followed by the same letter are not significantly ($P \leq 0.05$) different according to Duncan's New Multiple Range Test (n = 10).
+T.h = *Trichoderma harzianum*, T.v = *T. viride*, and B.s = *Bacillus subtilis.*Table (5) Effect of bio-control agents on some growth parameters of sour orange seedlings artificially infected with *T. semipenetrans* and *F. solani* under greenhouse conditions; 3 months after treatment (n = 10)*.

Bio-control agents[+]	***Growth parameters***							
	Shoots				***Roots***			
	Length (cm.)	**Increase (%)**	**Fresh weight (g)**	**Increase (%)**	**Length (cm.)**	**Increase (%)**	**Fresh weight (g)**	***increase (%)***
Untreated check	44.5 a	-	28.6 a	-	56.2 a	-	20.3 ab	-
F. solani (FS)	37.0 a	-	12.2 a	-	30.7 a	-	12.1 b	-
T. semipenetrans (TS)	40.7 a	-	13.8 a	-	36.7 a	-	11.0 b	-
N + F	44.5 a	-	13.0 a	-	33.2 a	-	10.6 b	-
T.h + TS + FS	41.2 a	-	17.3 a	-	42.7 a	-	16.1 ab	-
T.v TS + FS	39.7 a	-	24.1 a	-	33.5 a	-	14.7 ab	-
B.s + TS + FS	36.5 a	-	23.8 a	-	54.5 a	-	19.9 ab	-
Compost +T.v + TS + FS	51.5 a	15.7	19.1 a	-	41.0 a	-	15.7 ab	-
Compost +T.h + TS + FS	44.0 a	-	19.0 a	-	44.2 a	-	15.7 ab	-
Compost + B.s + TS + FS	43.2 a	-	16.9 a	-	38.7 a	-	24.6 ab	*21.2*
Compost +Tv + Th + (FS + TS)	44.0 a	-	19.1 a	-	54.5 a	-	19.9 ab	-
Compost + TS + FS	*48.5 a*	*9.0*	*26.7 a*	-	*42.7 a*	-	*28.6 a*	*40.9*

[*]Nematode-initial population = 3000 J_2 + males. Means in a column followed by the same letter are not significantly ($P \leq 0.05$) different according to Duncan's New Multiple Range Test (n = 10).
[+]T.h = *Trichoderma harzianum*, T.v = *T. viride*, and B.s = *Bacillus subtilis.* [(-)] means no increase.

The highest reduction was obtained with compost + *T. harzianum* or *T. viride* which reduced disease incidence and severity by 75%. All treatments significantly reduced the total count of *F. solani* in rhizospheric soil. The highest reduction in total count of *F. solani* was obtained with compost + mixture of *T. harzianum* + *T. viride* which reduced total count by 82.1% (Table 3). The highest increase in enzyme activities was obtained with combined treatments of compost and *T. harzianum*, *T. viride*, *B. subtilis* or (*T. harzianum* + *T. viride*) which increased the peroxidase, polyphenol oxidase and chitinase activities more than 300.0, 72.2 and 109.9 % respectively (Table 6).

These results are in partial or full agreement others [13,42,43,44.] In this respect, [44] found that the mechanisms of the antagonism of *T. harzainum* against different pathogens may be due to mycoparasitism, competition and antibiosis.

Utilization of composts to minimize organic waste pollution and to reduce the addition of chemical fertilizers, nematicides and fungicides in crop production is a promising strategy for both the present and the future and so can serve for sustainable agriculture [8]. Furthermore, many soil-borne pathogens can be reduced by application of composts made of different raw materials [15,27] and mature composts can sustain biological control agents [45]. In the present work, results revealed that the most effective treatment was compost + mixture of *T. harzianum* + *T. viride* which reduced disease incidence and severity on sour orange by 87.5%.

Table (6) Enzyme activities of sour orange seedlings in response to different treatments (n = 10)*.

Soil treatment+	Enzyme activities					
	Peroxidase		Polyphenol oxidase		Chitinase	
	Activity	Increase %	Activity	Increase %	Activity	Activity
Fusarium solani (FS)	0.5 d	—	1.5 d	—	1.2	—
Tylenchulus semipenetrans (TS)	0.7 d	—	1.7 d	—	1.0 d	—
FS + TS	0.7 d	0.0	1.8 d	0.0	1.1 d	0.0
Trichoderma harzianum (Th)+FS+TS	2.5 b	226.8	2.8 b	55.5	2.4 b	118.2
Trichoderma viride (Tv) + (FS +TS)	2.4 b	242.9	2.8 b	55.5	2.6 b	136.4
Bacillus subtilis (Bs) + (FS + TS)	2.3 b	228.5	2.2 c	22.2	2.0 c	81.8
Compost +Th + (FS + TS)	2.8 a	300.0	3.4 a	88.8	3.4 a	209.1
Compost +Tv + (FS + TS)	2.9 a	314.3	3.4 a	88.8	3.2 a	190.9
Compost +Bs + (FS + TS)	2.8 a	300.0	3.1 a	72.2	3.2 a	190.9
Compost +Tv + Th + (FS + TS)	2.9 a	314.3	3.5 a	94.4	3.6 a	227.3
Compost + (FS + TS)	1.4 c	100.0	2.4 c		2.7 b	100.0

*Nematode-initial population = 3000 J_2 + males. Means in a column followed by the same letter are not significantly ($P \leq 0.05$) different according to Duncan's New Multiple Range Test (n = 10).
+T.h = *Trichoderma harzianum*, T.v = *T. viride*, and B.s = *Bacillus subtilis.*

The incidence of several soil-borne plant pathogens, including phytonematodes, has also been reduced by using composts made of different raw materials [11,46,47,48]. In this respect, [25] reported that *Trichoderma* sp. in combination with composts from agricultural wastes, was used to suppress *Rhizoctonia solani* in cucumber seedlings. Also, *Trichoderma asperellum* with compost were used to suppress Fusarium wilt of tomato [47]. Our study documented the beneficial effects of biocontrol agents such as *Trichoderma harzianum, T. viride*, and *Bacillus subtilis* as well as organic amendments on suppressing *Tylenchulus semipenetrans* population densities and enhancing growth parameters of citrus seedlings [49,50]. Mature composts are known to sustain biological control agents, whereas immature composts do not. Immature composts also can negatively affect the growth of crop plants if they introduce pathogens to the soil or growing medium [3,4,43,45,49,50].

Currently, it is believed that a combination of antagonistic microbes with mature compost may be more efficient in inhibiting diseases than using single antagonistic microbial strain or compost alone [11,25,26,47].

Control of *T. semipenetrans* and *F. solani* through soil amended by organic materials or agricultural wastes alone or in combined with bio-control agents may be attributed to: 1-increasing the activity of the indigenous microflora resulting in suppressing pathogen populations through competition or specific inhibition, 2-releasing degradation compounds such carbon dioxide, ammonia, nitrites, saponins or enzymes which are generally toxic to the pathogens, 3-inducing plant defense mechanisms, 4- enhancing cellulase and glucanase to high concentration as a result of the breakdown of cellulase and lignin by microorganisms in the soil [51,52].

The reduction in *F. solani* infection and severity as well as *T. semipenetrans* rate of build-up on seedlings may be attributed to their decreased population densities in the soil and plant roots (Tables 2-4). The inhibitory effect of biocontrol agents might be related mainly to the antagonistic properties, which involve parasitism and lysis of pathogenic fungi and nematodes and/or competition for limiting factors in the rhizosphere, mainly iron and carbon [53,54]. Recently however, another possible mechanism has been suggested by [55] namely, induced resistance in plants to the attacking pathogens**.** Such a mechanism was supported in the present study by increasing enzyme activities in the infested plants treated with these bioagents and/or compost (Table 6). [18,56] reported that *Bacillus subtilis* isolates exhibited strong antagonistic activity against *M. phaseolina* and other phytopathogens including *F. oxysporum* and *R. solani*. [57] recorded that 38% of *B. subtilis* isolates showed competitive activity against *Fusarium oxysporum*. On the other hand, [6,11,19,58] reported that *T. viride, T. harzianum, T. hamatum* had ability to suppress growth of several plant-parasitic nematode species; e.g. *Meloidogyne* spp., and *T. semipenetrans* and fungal plant pathogens including *M. phaseolina*, *F. oxysporum* and *R. solani* on several economically important crops.

Our main goal was to document that the tested bioagents and/or compost can suppress these two pathogens simultaneously after such a relatively short period of time; 90 days. Yet, for more extended time, it is likely that other plant growth parameters, in addition to the increased fresh weight of roots (Table 5), would have benefited from such suppressions for both pathogens. Further investigations are necessary to confirm the effectiveness of the bioagents and/or compost we tested on *Fusarium solani* and *Tylenchulus semipenetrans* under field conditions. Controlling of plant diseases depends mainly on fungicides and nematicides application such chemicals are not always desirable due to potential hazards to human beings and the environment. . Alternative approaches to fungicides and nematicides are needed for controlling plant diseases [59, 60,61,62,63,64,65.]

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References

1. El .Mohamedy, R.S.R. (1998) .Studies on wilt and root rot disease of some citrus plants in Egypt .Ph. D. Thesis, Fac. Agric. Ain Shams Univ, pp. 227.
2. Catara, A. and G. Polizzi (1999). Dry root rot of citrus: symptoms, causing and susceptibility of rootstocks. Rivisto di Fruticolture 6 (11):38-41.
3. Duncan, L.W. (2005). Nematode parasites of citrus. In: Luc, M., Sikora, R.A. & Bridge, J. (Eds). *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, 2nd edition. Wallingford, U.K., CABI Publishing, pp. 437-466.
4. Verdejo-Lucas, S. and McKenry, M.V. (2004). Management of the citrus nematode, *Tylenchulus semipenetrans*. *Journal of Nematology* 36(4), 424-432.
5. Abd-Elgawad, M.M.M., Al-Yahya, F.A. & Stephan, Z.A. (2010). Nematodes of citrus. In: Abu-Gharbieh, W.A., Al-Hazmi, A.S., Stephan, Z.A. & Dawabah, A.A. (Eds). *Plant Nematodes in Arab Countries* (in Arabic). Amman, Jordan, Darwael for publishing, Arab Society of Plant Protection, pp.553-602.
6. Abd-Elgawad, M. M. M., and Kabeil, S. S. A. (2012) Biological control of *Meloidogyne incognita* by *Trichoderma harzianum* and *Serratia marcescens* and their related enzymatic changes in tomato roots. African Journal of Biotechnology 11:16247-16252.
7. Abd-Elgawad, M.M.M., Abd-El-Khair, H., Koura, Faika F. H., Abd El-Wahab, A.E., Montasser, S. A. and Hammam, M.M.A. (2013) Comparative effects of entomopathogenic nematodes and other biorational compounds on *Tylenchulus semipenetrans* Cobb populations on citrus. Egyptian Journal of Agronematology 12(1):74-90.
8. Abd-Elgawad, M.M.M. and Askary, T.H. (2015) Impact of phytonematodes on agriculture economy. In: Askary, T.H. and Martinelli, P.R.P. (eds) Biocontrol Agents of Phytonematodes. Wallingford, CAB International, UK, pp. 3-49.
9. Verma, K.S., S. Nartey and N. Singh (1999) Occurrence and control of dry root rot of citrus seedlings. Plant Disease Research 14 (2):31-34.
10. Abdel-Kader, M.M., Hammam, M.M.A., El-Mougy, N.S., Abd-Elgawad, M.M.M. (2015) Pesticide alternatives for controlling root rot and root knot of cucumber under plastic house. International Journal of Engineering and Innovative Technology 4(11), 25-31.
11. Askary, T.H. and Martinelli, P.R.P. (2015) Biocontrol Agents of Phytonematodes. Wallingford, CAB International, UK, 470 pp.
12. El-Mohamedy, R.S.R.; Abd –Alla, M.A. and Badiaa, R.I. (2006). Soil amendment and seed bio-priming treatments as alternative fungicides for controlling root rot diseases on cowpea plants in Nobria province. Research J. Agric and Biological Sci. (Pakistan) 2(6):391-398.
13. El-Mohamedy, R.S.R., El- Sayed H. Ziedan and Abd –Alla, M.A. (2010). Biological soil treatment with *Trichoderma harzianum* to control root rot disease of grapevine (*Vitis vinifera* L.) in newly reclaimed lands in Nobaria province. Archives of Phytopathology and plant protection 1:1-12.
14. El–Mohamedy, R.S.R., Morsey, A.A., Diab M.M., Abd- El-Kareem, F. and. Faraag , E.S. (2012a) Management of dry root rot disease of mandarin (*Citrus reticulate* Blanco) through bio composted agricultural wastes. J. Agricultural Technology. (3)969-981.

15. El-Mohamedy, R.S. M.M. Abdel-Kader, F. Abd-El-Kareem, M.A. Abd-Allah, El-Gamal, N.G. and Y.O. Fatouh (2012b) Field application of bio compost to control *Fusarium* dry rot disease of potato in newly Reclaimed lands. J. Agricultural Technology. (4): 1375-1387.
16. Eissa, M.F.M. and Abd-Elgawad, M.M.M. (2015) Nematophagous bacteria as biocontrol agents of phytonematodes. In: Askary, T.H. and Martinelli, P.R.P. (eds) Biocontrol Agents of Phytonematodes. Wallingford, CAB International, UK, pp. 217-243.
17. Abd-Elgawad, M.M.M. and Vagelas, I.K. (2015) Nematophagous bacteria: field application and commercialization. In: Askary, T.H. and Martinelli, P.R.P. (eds) Biocontrol Agents of Phytonematodes. Wallingford, CAB International, UK, pp. 276-309.
18. Singh, N.; Pandey, P.; Dubey, R.C. and Maheshwar, D. K. (2008) Biological control of root rot fungus *Macrophomina phaseolina* and growth enhancement of *Pinus roxburghii* Sarg. by rhizosphere competent *Bacillus subtilis* BN1. World J. Microbiol. Biotechnol. 24:1669-1679.
19. Tran, N. H. (2010) Using *Trichoderma* species for biological control of plant pathogens in Viet Nam. Phytopathol., 16:17-21.
20. Killani, A. S.; Abaidoo, R. C. 1.; Akintokun, A. K. and Abiala, M. A. (2011) Antagonistic effect of indigenous *Bacillus subtilis* on root-/soilborne fungal pathogens of cowpea. Researcher, 3:11-18.
21. Abdel-Kader, M. M.; El-Mougy, Nehal S.; Aly, M. D. and Lashin, S. M. (2012) Different approaches of biocontrol agents for controlling root rot incidence of some vegetables under greenhouse conditions. Inter. J. of Agric. and Forestry, 2:115-127.
22. Compant, S.; Muzammil, S.; Lebrihi, A. and Mathieu, F. (2013). Use of beneficial bacteria and their secondary metabolites to control grapevine pathogen diseases. BioControl, 58:435-455.
23. El-Naggar, M. A.; Abouleid, H.; Abdel-Kareem, F.; El-Deeb, H.M. and El-Shahawy, I.E. (2016a) Biological Control of Potato Late Blight by Means of Induction Systemic Resistance and Antagonism . Research Journal of Pharmaceutical, Biological and Chemical Sciences 7: 1338- 1348.
24. El-Naggar, M. A.; Abouleid, H.; Abdel-Kareem, F.; El-Deeb, H.M. and El-Shahawy, I.E. (2016b) Soft Rot Disease Management of Imported Potato Designed for Cultivation During Early Summery Season in Egypt. Research Journal of Pharmaceutical, Biological and Chemical Sciences 7: 1349- 1359.
25. Trillas, M.I.; Casanova, E.; Corxarrera, L.; Ordovas, J.; Borrero, C. and Aviles, M. (2006) Composts from agricultural waste and the *Trichoderma asperellum* strain T-34 suppress *Rhizoctonia solani* in cucumber seedlings. *Biol Control* , 39:32–38.
26. Abd-El-Kareem, F.; El-Mougy, N, S. and Abd-El- Kader, M.M. (2013) Application of compost and bio-agents as integrated soil treatment for controlling peanut crown rot disease under field conditions. *Advances in Agriculture, Sciences and Engineering Research*.3 (5) May: 858 – 866.
27. Zhao, S.; Liu, D.; Ling, N.; Chen, F.; Fang, W. and Shen, Q. (2014) Bio-organic fertilizer application significantly reduced the *Fusarium oxysporum* population and alters the communities of watermelon Fusarium wilt rhizosphere soil. Biol. Fertil. Soil, On Line, DOI: 10. 1007/00374-014.
28. John, P.R.; Tyagi, R.D.; Prevost, D.; Pouleur, S. and Surampalli, R.Y. (2010) Mycoparasitic *Trichoderma viride* as a biocontrol agent against *Fusarium oxysporum* f. sp. *adzuki* and *Pythium arrhenomanes*and as a growth promoter of soybean. Crop Protection, 29: 1452-1459.
29. Mansoori, M.; Nader, A.; Rezaee, S. and Naraghi, l. (2013) Evaluation of Pseudomonas and Bacillus bacterial antagonists for biological control of cotton Verticillium wilt Disease. Journal of Plant Protection Research. 53: 154–157.
30. Morsy, Ebtsam M.; Abdel-Kawi, K. A. and Khalil, M. N. A. (2009) Efficiency of Trichoderma viride and Bacillus subtilis as biocontrol agent against Fusarium solani on tomato plants. Egypt J. Phytopathol., 37:47-57.
31. Elad Y, Baker R (1985) Influence of trace amounts of cations and siderophore-producing Pseudomonas on chlamydospore germination of Fusarium oxysporum. Phytopathgol., 75: 1047–1052.
32. Abd-Elgawad, M.M.M., Duncan, L.W., Koura, F.H.F., Abd El-Wahab, A.E., Montasser, S.A. & Hammam, M.M.A. (2011) Management revision and observations on Tylenchulus semipenetrans on citrus yield in Egypt. Egyptian J. Agronematology 10(1), 64-77.
33. Korayem, A.M. & Hassabo, S.A.A. (2005) Citrus yield in relation to Tylenchulus semipanetrans in silty loam soil. International Journal of Nematology 15(2), 179-182.
34. Inserra, R.N.; Vovlas, N.; O'Bannon, J.H. and Esser, R.P. (1988) Tylenchulus graminis n. sp. and T. palustris n. sp. (Tylenchulidae) from native flora of Florida, with notes on T. semipenetrans and T. furcus. Journal of Nematology 20: 266-287.

35. Morgan, K.T. and L.W. Timmer (1984) Effect of inoculum density, nitrogen source and saprophytic fungi on Fusarium wilt of Mexican lime. Plant and Soil 79:203-210.
36. Papavizes, G.C. and R.D. Lumsden (1982) Improved medium for isolation of Trichoderma spp from soil. Plant Soil 66:1019–1020.
37. Goldschmidt, E.E.; Goren, R. and Monselise, S.P. (1968) The IAA oxidase system of citrus roots. Planta, 72: 213-222.
38. Allam, A.L. and Hollis, J.P. (1972) Sulfide inhibition of oxidase in rice root. Phytopathology, 62:634-639.
39. Matta, A. and Diamoned, C. (1963) Symptoms of Fusarium wilt in relation to quantity of fungus and enzyme activity in tomato stems. Phytopathology, 53: 574-587.
40. Monreal. J. and Reese, E.T. (I969) The chitinase of Serratia marcescens. Canadian J. of Microbiology, 15: 689-696.
41. Abd-Elgawad, M. M. (1992) Spatial distribution of the phytonematode community in Egyptian citrus groves. Revue De Nematologie 14:367-373.
42. EL.Mohamedy, R.S.R. (2004) Bio-priming of okra seed to control damping-off and root rot diseases. Annals Agric. Sci. Ain Shams Univ.Cairo.49 49(11):339-356.
43. Abd-Elgawad, M.M.M., M.H. Abou-Deif, M.M.A. Hammam, H. Abd-El-Khair, Faika F. H. Koura, A. E. Abd El-Wahab and S. A. Montasser (2015) Effect of infection with Tylenchulus semipenetrans on enzymatic activities in citrus. International Journal of Engineering and Innovative Technology 4(12): 43-48.
44. Elad T., Chet J. and Katan J. (1980) Trichoderma harzianum a biocontrol effective against Sclerotium rolfsii and Rhizoctonia solani. Phytopathology 70:119-121.
45. Litterick, A.; Harrier, L.; Wallace, P.; Watson, C. and Wood, M. (2004) The role of uncomposted materials, composts, manures and compost extracts in reducing pest and disease incidence and severity in sustainable temperate agricultural and horticultural crop production. Rev Plant Sci., 23:453–479.
46. Borrero, C.; Trillas, M.I.; Ordova, J.; Tello, J.and Avile, M. (2004) Predictive factors for the suppression of Fusarium wilt of tomato in plant growth media. Phytopathology, 94:1094–1101.
47. Cotxarrera L, Trillas-Gay, M.I.; Steinberg, C. and Alabouvette, C. (2002) Use of sewage sludge compost and Trichoderma asperellum isolates to suppress Fusarium wilt of tomato. Soil Biol Biochem 34:467–476.
48. Abdel-Kader, M.M.; Abd-El-Karem, F.; El-Mougy, N, S. and El- Mohamedy, R.S. (2013) Integration between compost, Trichoderma harzianum and essential oils for controlling peanut crown rot underfield conditions. Journal of Mycology, 2013, Article ID 262130, 7 pages.
49. Montasser, S.A., Abd El- Wahab, A.E., Abd-Elgawad, M.M.M., Abd-El-Khair, H., Koura, Faika, F.H. and Hammam, M.M.A. (2012a) Role of some plant extracts and organic manure in controlling Tylenchulus Semipenetrans Cobb in vitro and in vivo in citrus. Journal of Applied Sciences Research, 8(11): 5415-5424.
50. Montasser, S.A., Abd El- Wahab, A.E., Abd-Elgawad, M.M.M., Abd-El-Khair, H., Koura, Faika, F.H. and 2Hammam, M.M.A. (2012b) Effects of some fungi and bacteria as bio-control agents against citrus nematode Tylenchulus Semipenetrans Cobb. Journal of Applied Sciences Research, 8(11): 5436-5444.
51. Walker, G.E. and B.G. Morry (1999) Effect of brassica and weed manures on abundance Tylenchulus sempleaetrans and fungi in citrus orchard soil. Australian Journal Exp. Agric. 38:65-72.
52. Liu, C.H. and J.W. Huany (2000) Effect of soil amendment of FBN- SA mixture on control of radish yellows and its possible mechanisms for inhibition of the pathogen. Pant Protection Bulletin Tapil 42:169-182.
53. Velazhahan, R.; Samiyappan, R. and Vidhyasekaran, P. (1999) Relationship between antagonistic of Pseudomonas fluorescens strains against Rhizoctonia solani and their production of lytic enzymes. J. Plant Dis. Prot. 106:244-250.
54. Wahyudi, A. T.; Astuti, R. P.; Widyawati, A.; Meryandini, A. A. and Nawangsih, A. A. (2011) Characterization of Bacillus sp. strains isolated from rhizosphere of soybean plants for their use as potential plant growth for promoting rhizobacteria. J. of Microbiol. and Antimicrob., 3:34-40.
55. Baraka, M. A.; Shaban, W. I; Awad, Nemat M. and Zian, A. H. (2012) Induction of systemic resistance and growth promotion by selected strains of rhizobacteria against lupine fusarium wilt. Egypt. J. Phytopathol., 39(2):107-122.
56. Vethavalli, S. and Sudha, S. S. (2012) In vitro and in silico studies on biocontrol agent of bacterial strains against Fusarium oxysporum f. sp. lycopersici. Res. Biotechn. 3:22-31.

57. Archana, G.; Alok, R. R.; Sudhir, U. M. and Dongre, A. B. (2010) Isolation, evaluation and characterization of Bacillus subtilis from cotton rhizospheric soil with biocontrol activity against Fusarium oxysporum. World J. Microbiol Biotechnol., 26:1187-1194.
58. Chakraborty, U. and Chatterjee, N. C. (2007) Interaction of Trichoderma harzianum with Furarium solani during its pathogenesis and the associated resistance of the host. Asian J. Exp. Sci., 21:353-357.
59. Elshahawy, I.E. Lashin, S.L. ; Saied, N.M. and Abd-El-Kareem, F. 2015. Evaluation of safe postharvest treatments for controlling Valencia orange green and blue moulds. International Journal of ChemTech Research, 8, No.9 : 237-244 .
60. Mawardika, H. and Suharjono, S. 2015. Antagonist Assay and Molecular Identification of Soil Molds Antagonist to Pathogenic Fusarium on Tomato Plants (Lycopersicum esculentum Mill.) in Bocek East Java Tomato Field. International Journal of ChemTech Researchm, 8, No.8,: 01-07.
61. Hathout, A.N. ; Abo-Sereih, N. A. ; Sabry, B.A. ; Sahab, A.F. and Aly, S.A. 2015. Molecular identification and control of some pathogenic Fusarium species isolated from maize in Egypt. International Journal of ChemTech Researchm, 7, No.1,: 44- 54.
62. Ghoname, A.A. ; Riad, G.S. ; El-basiouny, A.M. ; Hegazi, A.M. and El-Mohamady, R.S. 2015. Finding natural alternatives to methyl bromide in greenhouse cantaloupe for yield, quality and disease control. International Journal of ChemTech Research, 8, No.9: 84-92.
63. Abdalla M.Y.; Haggag, W.M. and Rayan, M.M.2015 . Using Bioproducts Made with Native Microorganisms to Limit the Damage for Some Sugar Beet Cultivars Seeds. International Journal of ChemTech Research, 8, No.9: 245-260,
64. Vimala Kumari, T.G.; Basu, K.; Nithya, T.G. ; and Kharkwa, K.S. 2015. Study of Bio-efficacy of Alkali tolerant Trichoderma against damping off and rotting diseases of Tomato and Cauliflower caused by Pythium spp. and Sclerotina spp. . International Journal of ChemTech Research, 8, No.6: 628-634.
65. El-Sayed, S. M. and Mahdy, M. E.2015. Effect of chitosan on root-knot nematode, Meloidogyne javanica on tomato plants. International Journal of ChemTech Research, 7, No.4 : 1985-1992.

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