

Evaluation of soil Amended with Bio-Agents and Compost Alone or in Combination for Controlling Citrus nematode *Tylenchulus semipenetrans* and Fusarium Dry root rot on Volkamer lime Under Greenhouse Conditions

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Abstract : The efficiency of soil amended with bio-agents and/or compost to control Fusarium dry root rot disease and citrus slow decline of Volkamer lime seedlings under greenhouse conditions was investigated. All tested bioagents reduced *Tylenchulus semipenetrans* population densities and the linear growth of *Fusarium solani*. The most effective treatment against *F. solani* was compost + mixture of *Trichoderma harzianum* + *T. viride* which reduced disease incidence and severity by 83.3 and 87.5%, respectively. The highest reduction was obtained with compost + *T. harzianum* or *T. viride* which reduced disease incidence and severity by 66.7 and 75% respectively. Infested soil treated with compost + *T. harzianum* or *T. viride* resulted in reducing total count of *F. solani* more than 61.2%. The highest increase in enzyme activities was obtained with mixture of compost and *T. harzianum*, *T. viride*, *Bacillus subtilis* or (*T. harzianum* + *T. viride*) which increased the peroxidase, polyphenol oxidase and chitinase activities by more than 65.2%. The compost with each of *B. subtilis*, *T. harzianum* or *T. viride* could reduce the rate of nematode build-up to 0.38, 0.40, and 0.42; respectively. In the presence of both pathogens *T. semipenetrans* and *F. solani*, treatment with compost alone could increase ($P \leq 0.05$) shoot length of Volkamer lime over that treated with *T. viride*. Other treatments were less effective.

Key words: Dry root rot disease, citrus trees, *Fusarium solani*, *Tylenchulus semipenetrans*, bio-agents, ompost, Volkamer lime, greenhouse.

Introduction

Dry root rot and slow decline diseases of citrus caused by *Fusarium solani* (Mart.) and *Tylenchulus semipenetrans* (Cobb), respectively, are serious diseases attacking many groves causing considerable losses in Egypt and elsewhere¹⁻⁸. Both pathogens can infect and thrive on Volkamer lime; a common rootstock, only after sour orange, in Egypt so far.

Control of these two pathogens relies mainly on chemical application^{9,5}. Due to the side effects of such chemicals, current experimentations of environmentally friendly alternatives are in progress with a clear aim at the avoidance of health and environmental hazards.^{10,11,12,13,14} on economically important crops¹⁵⁻²⁰.

Although voluminous work has been done on such alternatives²¹⁻²⁷ information is scanty on the impact of such alternatives on these two pathogens infecting Volkamer lime (*Citrus volkamerina* Tenet Pas.)

Therefore, the present work evaluated the efficiency of soil amended with bio-agents and/or compost to control the citrus slow decline and fusarium dry root rot diseases under greenhouse conditions. Enzymatic activities of peroxidase, polyphenol oxidase and chitinase, that may be included in inducing plant resistance to such attacking pathogens, were also assessed.

Materials and Methods

Source of pathogenic fungus, bio-agents and plant material:

Pathogenic isolate of *F. solani* the causal agent of dry root rot disease of citrus plants and the antagonistic strains of *Trichoderma harzianum*, *T. viride* and *B. subtilis* was obtained from Plant Pathol. Dept., NRC. The Volkamer lime (*Citrus volkamerina* Tenet Pas.) seedlings were obtained from Citrus Research Division, Agriculture Reseach Center, Giza, Egypt.

Source of plant compost :

Plant compost obtained from El- Nile Company, Giza, Egypt (Table 1).

Table 1: Chemical analysis and properties of the plant compost used in the study.

Total Nitrogen (%)	1.53
Total Phosphorus (%)	0.29
Total Potassium (%)	1.13
Total Iron (ppm)	1368
Total Zinc (ppm)	70.0
Total Copper (ppm)	12.0
Total organic matter (%)	31.0
Organic Carbon (%)	33.4
Carbon / Nitrogen ratio	12:1
pH (1:100)	8.1
EC	1:100
Humidity (%)	8.54

Greenhouse experiment

1) Impact of bio-agents and/or compost on the two pathogens and seedlings:

This experiment was carried out to evaluate the efficacy of bio-agents and/or compost against *Fusarium solani* and *Tylenchulus semipenetrans* under greenhouse conditions.

Preparation of Biocontrol agent inocula:

T. viride and *T. harzianum* were grown and cultured according to ²⁸. Antagonistic bacterial cells were then collected and centrifugated at 6,000 rpm for 15 min, re-suspended in a phosphate buffer (0.01 M, pH 7.0), and adjusted by plate count technique to an approximately 3×10^7 cfu/ ml as mentioned by ^{28,17,18}.

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 ²⁹. One 2-year-old healthy seedling of the common rootstock in Egypt, *i.e.*, Volkamer lime was transplanted to each pot.

Preparation and inoculation of *Tylenchulus semipenetrans*

Soil and root subsamples were obtained from 'Baladi' mandarin (*Citrus reticulata* Blanco) orchard ³⁰ 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 cm³ 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-cm³ 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 nematode³¹. Key reference of³² was consulted to identify *T. semipenetrans* via examining several mounted adult females in glycerin. All counted live *T. semipenetrans* J2 and males were returned back to the bulk soil which was calibrated 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. viride* or *T. harzianum* (10¹² cfu/ml) in addition to the antagonistic bacteria, *i.e.* *B. subtilis*, (10¹⁶ cfu/ml) were prepared as previously mentioned. 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 (10⁶ cfu/ml)/ kg soil of each antagonistic fungi or bacteria. Plant compost or bio-compost (compost + bio agents) was added to soil infested with *T. semipenetrans* and *F. solani* at the rate of 50 g/ kg soil. The compost and/or bioagent(s) were added 5 days after nematode inoculation.

Treatments

The treatments were 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*
12. Soil infested with (FS + TS)+ PC+ *T. harzianum*+ *T. viride*

Ten replicates/pots were used for each treatment

Disease assessment:

Ninety days after treating Volkamer lime 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 to³³. 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 to³⁴ 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 Volkamer lime 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 Volkamer lime seedlings.

Extraction of enzymes: Volkamer lime 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)³⁵. 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 by³⁶. 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% H₂O₂ 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 by³⁷. 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 of³⁸.

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. semipenetrans* showed contagious distribution³⁹.

Results

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

Treatment effects on root rot disease of Volkamer lime

Results in Table (2) showed that the most effective treatment was compost + mixture of *T. harzianum* + *T. viride* which reduced disease incidence and severity by 83.3 and 87.5% respectively. The highest reduction was obtained with compost + *T. harzianum* or *T. viride* which reduced disease incidence and severity by 66.7 and 75.0% respectively. Other treatments were less effective.

Table 2. Effect of biological soil treatment on root rot complex of Volkamer lime caused by *Fusarium solani* and *Tylenchulus semipenetrans* under greenhouse conditions (n = 10)*.

Soil treatment	Root rot disease incidence			
	Disease infection	Reduction %	Disease severity	Reduction %
<i>Fusarium solani</i> (FS)	40 b	—	35 abc	—
<i>Tylenchulus semipenetrans</i> (TS)	—	—	—	—
FS + TS	60 a	0.0	40 a	0.0
<i>Trichoderma harzianum</i> (Th) + (FS + TS)	40 c	33.3	25 cd	37.5
<i>Trichoderma viride</i> (Tv) + (FS + TS)	40 c	33.3	25 c d	37.5
Bacillus subtilis (Bs) + (FS + TS)	50 b	16.7	30 c	25
Compost +Th + (FS + TS)	20 d	66.7	10 e	75
Compost +Tv + (FS + TS)	20 d	66.7	10 e	75
Compost +Bs + (FS + TS)	40 c	33.3	20 d	50
Compost +Tv + Th + (FS + TS)	10 e	83.3	5 e	87.5
Compost + (FS + TS)	40 c	33.3	20 d	50
Untreated check	0.0 f	0.0	0.0 f	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.

Effect of bioagents on total count of *Fusarium solani* in Volkamer lime rhizospheric soil.

Results in Table (3) showed that 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 88% with Volkamer lime, respectively. Infested soil treated with compost + *T. harzianum* or *T. viride* resulted in reducing total *F. solani* by more than 61.2%. Other treatments were less effective.

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

Soil treatment	Average propagules of <i>F. solani</i> (cfu X 10 ⁵ / g dry soil)	
	Volkamer lime	
	Total count	Reduction %
<i>F. solani</i> (FS)	42.4	
<i>T. semipenetrans</i> (TS)	—	—
FS + TS	48.4 a	0.0
<i>T. harzianum</i> (Th) + FS + TS	15.4 de	68.2
<i>T. viride</i> (Tv) + (FS + TS)	18.8 d	61.2
Bacillus subtilis (Bs) + (FS + TS)	26.2 c	45.9
Compost +Th +(FS + TS)	8.2 f	83.1
Compost +Tv +(FS + TS)	9.8 f	79.8
Compost +Bs + (FS + TS)	13.4 e	69.1
Compost +Tv + Th +(FS + TS)	5.8 g	88.0
Compost +(FS + TS)	30.2 b	37.6

*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 Volkamer lime under greenhouse conditions after 3 months of treatments*.

Bio-control agents ⁺	Nematode parameters				Final population		Rate of nematode build up
	J ₂ in 250g soil		Roots		Count	Efficacy (%)	
			Female and Eggs (total) in 5g roots				
	Count	Reduction (%)	Count	Reduction (%)	Count	Efficacy (%)	
<i>T. semipenetrans</i> only (N)	570 a	----	3320 a	----	3890	----	1.3
N + <i>F. solani</i> (F)	386 bc	32.3	2652 ab	20.1	3038	21.9	1.3
T.h + N + F	373 c	34.6	2779 ab	16.3	3152	19.0	1.1
T.v + N + F	515 ab	9.6	1779 bc	46.4	3394	41.0	0.76
B.s + N + F	514 ab	9.8	847 c	74.5	1361	65.0	0.45
Compost + T.v + N + F	522 ab	8.4	740 c	77.7	1262	67.5	0.42
Compost + T.h + N + F	381 c	33.1	822 c	75.2	1203	69.0	0.40
Compost + B.s + N + F	379 c	33.5	752 c	77.3	1121	70.9	0.38

*Nematode-initial population = 3000 J₂ + 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 Volkamer lime 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	50.2 ab	-	22.8 a	-	58.5 a	-	19.6 a	-
<i>F. solani</i> (F)	36.0 ab	-	20.0 a	-	54.2 ab	-	20.4 a	4.1
<i>T. semipenetrans</i> only (N)	32.2 ab	-	23.8 a	4.4	42.2 ab	-	21.5 a	9.7
N + F	31.7 ab	-	27.5 a	20.6	46.0 ab	-	24.3 a	24.0
T.h + N + F	40.7 ab	-	21.5 a	-	47.5 ab	-	19.5 a	-
T.v + N + F	28.5 b	-	27.4 a	20.2	51.5 ab	-	22.8 a	16.3
B.s + N + F	40.7 ab	-	19.5 a	-	40.2 ab	-	17.0 a	-
Compost + T.v + N + F	46.0 ab	-	25.7 a	12.7	40.0 ab	-	20.7 a	5.6
Compost + T.h + N + F	52.5 ab	4.6	18.9 a	-	51.7 ab	-	16.4 a	-
Compost + B.s + N + F	41.0 ab	-	20.3 a	-	37.2 bc	-	16.6 a	-
Compost + (FS + TS)	55.5 a	10.5	22.6 a	-	33.2 c	-	16.5 a	-

*Nematode-initial population = 3000 J₂ + 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.

Evaluate the efficiency of soil amended with bio-agents and compost alone or in combination on enzyme activities of citrus seedlings.

Results in Table (6) indicated that all tested treatments significantly increased the tested enzyme activities. The most effective treatments were combination of compost and *T. harzianum*, *T. viride*, *B. subtilis* or (*T. harzianum* + *T. viride*) which increased the peroxidase, polyphenol oxidase and chitinase activities by 169.2, 65.2 and 85.7%, respectively. Single treatments were less effective.

Table (6) Enzyme activities of Volkamer lime plants in response to different bioagents and compost.

Soil treatment	Enzyme activities					
	Peroxidase		Polyphenol oxidase		Chitinase	
	Activity	Increase %	Activity	Increase %	Activity	Activity
<i>Fusarium solani</i> (FS)	1.0 e	—	1.8 d	—	1.5 d	—
<i>Tylenchulus semipenetrans</i> (TS)	1.2 e	—	2.0 d	—	1.8 d	—
FS + TS	1.3 e	0.0	2.3 d	0.0	2.1 d	0.0
<i>Trichoderma harzianum</i> (Th) + (FS + TS)	3.4 b	162.3	3.7 b	60.9	4.2 b	100.0
<i>Trichoderma viride</i> (Tv) + (FS + TS)	3.3 b	156.3	3.6 b	56.5	4.5 b	114.3
<i>Bacillus subtilis</i> (Bs) + (FS + TS)	2.9 c	123.1	2.8 c	21.7	4.0 c	90.5
Compost +Th + (FS + TS)	3.7 a	184.6	4.2 a	82.6	4.9 a	133.3
Compost +Tv + (FS + TS)	3.8 a	192.3	4.4 a	91.3	4.8 a	128.6
Compost +Bs + (FS + TS)	3.5 a	169.2	3.8 a	65.2	3.9 a	85.7
Compost +Tv + Th + (FS + TS)	3.8 a	192.3	4.2 a	82.6	5.0 a	138.9
Compost + (FS + TS)	2.3 d	153.8	2.8 c	21.7	2.8 c	33.3

*Nematode-initial population = 3000 J2 + 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*.

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^{1,5,30}. All tested treatments (Tables 2-6) 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 phytonematodes and various soil-borne plant diseases in many countries^{13,24,25,26,27}. In the present study, 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 83.3 and 87.5 % respectively.

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 88% with. 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 65.2%.

These results are in agreement with^{16,40,41} found that the mechanisms of the antagonism of *T. harzianum* 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 and fungicides in crop production is a promising strategy for both the present and the future. Furthermore, many soil-borne pathogens can be reduced by application of composts made of different raw materials^{12,17,18} and mature composts can sustain biological control agents⁴². In the present research, results revealed that the most effective treatment was compost + mixture of *T. harzianum* + *T. viride* which reduced disease incidence and severity by 87.5% on Volkamer lime.

The incidence of several soil-borne plant pathogens has also been reduced by using composts made of different raw materials^{11,43,44}. In this respect, ¹⁰ 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 was used to suppress Fusarium wilt of tomato⁴⁴. 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^{3,4,7,42,45,46,47}. Mature composts are known as better sustain biological control agents than immature composts. Immature composts can negatively affect the growth of crop plants if they introduce pathogens to the soil or growing medium⁴². 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^{44,10,11}.

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 suppression of pathogens population 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- cellulase and glucanase are prevalent to high concentration as a result of the breakdown of cellulase and lignin by microorganisms in the soil^{48,49}.

The reduction in both disease infection and severity on seedlings may be attributed to the high decrease in population density of the pathogen in the soil. The inhibitory effect of biocontrol agents might be related mainly to the antagonistic properties, which involve parasitism and lysis of pathogenic fungi and/or competition for limiting factors in the rhizosphere, mainly iron and carbon^{50,51}. Recently however, another possible mechanism has been suggested by⁵² namely, induced resistance in plants to the nematode and fungus attack.

^{21,53} reported that *Bacillus subtilis* isolates exhibited strong antagonistic activity against *M. phaseolina* and other phytopathogens including *F. oxysporum* and *R. solani*.⁵⁴ recorded that 38% of *B. subtilis* isolates showed competitive activity against *Fusarium oxysporum*. On the other hand, ^{55,22} reported that *T. viride*, *T. harzianum*, *T. hamatum* had ability to suppress growth of fungal plant pathogens including: *M. phaseolina*, *F. oxysporum* and *R. solani* on several 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 length of shoots (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^{56, 57, 58, 59, 60,61,62}

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