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USE OF MICROBIOAGENTS TO REDUCE SOIL PATHOGENS AND ROOT-KNOT NEMATODES IN GREENHOUSE-GROWN TOMATOES

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Abstract

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Single-crop greenhouse production of vegetables often results in the accumulation of pathogens and root-knot nematodes in the soil that threaten production. Recent efforts have been focused more efficient, environmentally sustainable and safe alternatives for controlling these pathogens. Pot experiments with tomatoes cv. Belle F_1 were conducted in the Maritsa Vegetable Crops Research Institute in Plovdiv under greenhouse conditions. They included bioagents in soil where seedlings were grown with and without compost. Microbial products *Bacillus thuringiensis* strain Bt1+*Bacillus amyloliquefaciens* strain 2/7A and bionematicide BioAct WG (*Paecilomyces lilacinus* strain 251) were added at three different stages to reduce root-knot nematodes (*Meloidogyne* spp.). The effects of soil pathogens *Fusarium oxysporum* f. sp. *lycopersici* and *Pyrenochaeta lycopersici* were reduced with microbial products *B. amyloliquefaciens* strain A1 and *Trichoderma viride* strain T6. The lowest root-galling rate was recorded in tomatoes grown with compost, both microbial products and bionematicide. The lowest degree of *Fusarium* wilt and corky root infestation was for trials grown with compost and the bioproduct *T. viride*. Improved biometrical plant indices were found in trials that used compost. Adding microbioagents in plant-protection schemes is an alternative that can control soil pathogens and root-knot nematodes under greenhouse conditions.

Key words: root-knot nematodes, soil pathogens, *Trichoderma*, *Bacillus*, compost

Introduction

Soil pathogens and root-knot nematodes cause serious damage in greenhouse-grown vegetables. Controlling them with chemical products creates a potential risk for environment and human health. Therefore biological control is one of the most promising alternatives (Kalele et al., 2010).

The control of soil pathogens in cultivation facilities is difficult because the collection of resistant cultivars and registered plant protection products (PPP) are insufficient. One of the most widespread bioagents with wide-spectrum action is the soil fungus *Trichoderma* spp. (Elad, 2000). Caron et al. (2002) has established that the strain MAUL-20 of *Trichoderma harzianum* reduces the manifestations of five pathogens (*Fusarium oxysporum* f. sp. *lycopersici*, *Pythium ultimum, Rhizoctonia solani, Sclerotinia sclerotiorum, Verticillium dahliae*) and stimulates cucumber growth and yield. In a study of compost that inhibits the growth of soil pathogens, Pugliese et al. (2014) isolated four accessions from *Trichoderma* spp. and three accessions of active bacteria, which successfully controlled pathogens. The bacterial strains appear to be more effective bioagents. The application of extracts from seaweed combined with bioagents (*T. harzianum* and *Bacillus subtilis*) significantly decreases root rot in cucumbers, tomatoes and peppers (Abdel-Kader and El-Mougy, 2013).

Biological agents suitable for control of the nematode populations and stimulating plant growth include rhizobacteria, parasitic bacteria and fungi (Sikora, 1992; Tian et al., 2007;

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Mokbel, 2013). Bacteria of the genera *Agrobacterium, Alcaligenes, Bacillus, Clostridium, Desulfovibrio, Pseudomonas, Serratia* and *Streptomyces* have a nematicidal effect (Siddiqui and Mahmood, 1999). *Pasteuria penetrans* directly parasitizes nematodes and *Bacillus*, *Agrobacterium*, *Azotobacter*, *Pseudomonas* and *Clostridium* produce toxins that kill them (Walia et al., 2000; Mohammed et al., 2008). *Bacillus* spp. decreases the infestation from root-knot nematodes (Mohammed et al., 2008; Mohamedova, 2009; Terefe et al., 2009). Some well-adopted commercial products contain the bacteria *B. thuringiensis*, *B. firmus, P. penetrans* and the fungus *Paecilomyces lilacinus* (Radwan, 2007; Lamovšek et al., 2013). They reduce nematode populations in the rhizosphere and some of them such as *B. amyloliquefaciens* promoted plant growth (Lobna and Zawam, 2010). Compost inhibits the effect of soil pathogens *Pythium* spp. (Pascual et al., 2000), *Phytophthora* spp. (Widmer et al., 1999), *Rhizoctonia* spp. (Tuitert et al., 1998) and *Fusarium* spp. (Suárez-Estrella et al., 2007). Sabet et al. (2013) consider that compost could be used as an effective means to control root rot in cucumbers caused by *F. solani*, *P. ultimum*, *Rh. solani*, and *Sclerotium rolfsii*. This is probably due to the microorganisms in compost that release enzymes and antibiotics or because they compete with pathogens for nutrients (Litterick et al., 2004).

During the last few years there has been increased interest in ecologically resistant and safe methods for controlling soil pathogens and root-knot nematodes. The application of microbioagents makes it possible to cultivate vegetables without pesticides.

The aim of the study was to establish the biological effects of fungal and bacterial bioagents on the soil pathogens *Fusarium oxysporum* f. sp. *lycopersici, Pyrenochaeta lycopersici* and root-knot nematodes *Meloidogyne* spp. in soil used to grow tomato seedlings in greenhouses. Trials were done with and without compost.

Materials and Methods

The experiments were conducted in 2013-2014 in the Maritsa Vegetable Crops Research Institute under greenhouse conditions with the tomato cv. Belle F_1.

Soil-borne diseases

The pathogens *F. oxysporum* f. sp. *lycopersici* and *P. lycopersici* were added to the containers after transplanting. The suspension of *B. amyloliquefaciens* strain A1 (titer 10^4 spores/1 cm^3 substrate) and *T. viride* strain T6 (titer 10^4 spores/1 cm^3 substrate) was added three times (while pricking out seedlings, transplanting and one month after transplanting) (Table 1).

The impact of pathogens was recorded on a five-point scale (0–4): 0 = no attacked, 1 = only single root affected, 2 = 25% of roots affected; 3 = 26-50% of roots affected and 4 = over 50% of roots affected.

Root-knot nematodes

Tomatoes were inoculated with 2000 second-stage juveniles (J2) in each 5 L container. Suspension from *Bacillus thuringiensis* strain Bt1 + *Bacillus amyloliquefaciens* strain 2/7A (titer 10^4 spores/1 cm^3 substrate) and BioAct WG (*Paecilomyces lilacinus* strain 251, titer $1x10^{10}$ spores per g product) was added three times (while pricking out seedlings, transplanting and one month after transplanting) – 0.2 g/plant (Table 2).

Root systems were rated for nematode-induced galling on a scale of 0–5: 0 = no galling, 1 = trace infections with a few small galls, 2 = < 25% roots with galls, 3 = 25-50% roots with galls, 4 = 50-75% roots with galls and 5 = > 75% roots with galls (Hussey and Janssen, 2002).

The plants were removed 60 days after transplanting and the following indices were recorded: shoot length (cm), fresh shoot weight (g), diameter of stem (mm), root length (cm) and fresh root weight (g).

Table 1
Test conditions 1

Trials with compost	Trials without compost
1. Control	Control
2. *Bacillus amyloliquefaciens* strain A1	*Bacillus amyloliquefaciens* strain A1
3. *Trichoderma viride* strain T6	*Trichoderma viride* strain T6
Pricking out seedlings in mixture: Peat:perlite:compost - 1:1:0.7	Pricking out seedlings in mixture: Peat:perlite:soil - 1:1:1

Table 2
Test conditions 2

Trials with compost	Trials without compost
1. Control	1. Control
2. *B. thuringiensis* strain Bt1 + *B. amyloliquefaciens* strain 2/7A (Bacterial strain)	2. *B. thuringiensis* strain Bt1 + *B. amyloliquefaciens* strain 2/7A (Bacterial strain)
3. BioAct WG	3. BioAct WG
Pricking out seedlings in mixture: Peat:perlite:compost - 1:1:0.7	Pricking out seedlings in mixture: Peat:perlite:soil - 1:1:1

The microorganisms mixed into the substrate, *B. thuringiensis* strain Bt1 and *B. amyloliquefaciens* strain 2/7A, had been tested previously for biological compatibility. It was established that being in contact together does not limit their growth.

The compost composition was 78% rye-grass and 22% farmyard manure with the following characteristics: pH = 7.88, EC = 3.61, N = 600 ppm, P = 12.0 ppm, K = 1547.6 ppm, Ca = 2100.0 ppm and Mg = 115.2 ppm.

Data were processed using three-way analysis of variance (Lidanski, 1988) and Duncan's multiple range test.

Results and Discussion

Soil-borne diseases

The three-way analysis of variance demonstrates significant impact (30.95%) of the Factor Variant (C) on the infestation rate of *Fusarium oxysporum* f. sp. *lycopersici.* The Factor B (Medium) and the interaction A*C exerts influence ($p < 0.05$) on the infestation rate but this effect was smaller (2.83% and 3.51%). The Factor Year (A) and interactions with the other factors had no significant effect on the infestation rate (Table 3).

Biometrical indices are the highest in the variants with *B. amyloliquefaciens*, according to the results of the trial infected with *F. oxysporum* f. sp. *lycopersici*. In this variant, shoot length (154.85 cm), fresh shoot weight (243.25 g), root length (38.81 cm) and root weight (37.32 g) were the highest (Table 4). Treatment with *Trichoderma viride* also stimulates plant growth. However, the differences between the variants are not statistically significant except for stem diameter and root weight. The values for these measures are higher for plants grown with compost. The infestation rate in this variant is lower only in the trial without compost and *T. viride* and in the variant with compost and *B. amyloliquefaciens*, they are proven (Figure 1).

The results confirm that the fungi of genus *Trichoderma* and bacteria reduce soil pathogens, as established by Pugliese et al. (2014). The effect of *T. viride* is stronger.

The results of *Pyrenochaeta lycopersici* for variant (C) are statistically significant (29.24%). The remaining factors

Table 3
Influence of experimental factors on the infestation rate of *Fusarium oxysporum* f. sp. *lycopersici*

Source	Sum of Squares	df	Mean Square	η%	Sig.
Year (A)	0.07	1	0.07	0.21	
Medium (B)	1.01	1	1.01	2.83	*
Variant (C)	11.02	2	5.51	30.95	***
A * B	0.01	1	0.01	0.02	
A * C	1.25	2	0.62	3.51	*
B * C	0.22	2	0.11	0.61	
A * B * C	0.12	2	0.06	0.33	

η% - Power of influence in %, *p<0.05; ** p<0.01; *** p<0.001.

Table 4
Effect of *Bacillus amyloliquefaciens* strain A1 and *Trichoderma viride* strain T6 on plant growth of tomato variety Belle F_1 infected with *Fusarium oxysporum* f. sp. *lycopersici*

Variants	Shoot length, cm	Fresh shoot weight, g	Diameter of stem, mm	Root length, cm	Fresh root weight, g
Without compost					
Control	150.30 ±24.23 n.s.	230.00±45.65 n.s.	7.74±0.59 d	36.05±16.06 n.s.	30.81±10.15 b
B. amyloliquefaciens	154.85 ±25.03 n.s.	243.25±65.74 n.s.	7.90±0.62 cd	38.81±14.25 n.s.	37.32±18.07 ab
T. viride	151.55 ±32.95 n.s.	231.75±59.05 n.s.	8.31±0.71 bc	37.24±16.99 n.s.	34.28±11.64 ab
With compost					
Control	141.20± 24.87 n.s.	245.50±46.25 n.s.	8.70±0.94 ab	31.59±9.92 n.s.	32.47±6.26 ab
B. amyloliquefaciens	147.60 ±21.66 n.s.	267.50±56.81 n.s.	9.08±0.76 a	32.71±6.97 n.s.	39.09±12.31 a
T. viride	152.90 ±26.14 n.s.	263.75±77.49 n.s.	8.82±0.72 a	37.39±13.65 n.s.	33.43±7.43 ab

a, b, c …n.s. – Duncan's multiple range test ($p < 0.05$).

and interactions have no significant effect on the infestation rate (Table 5).

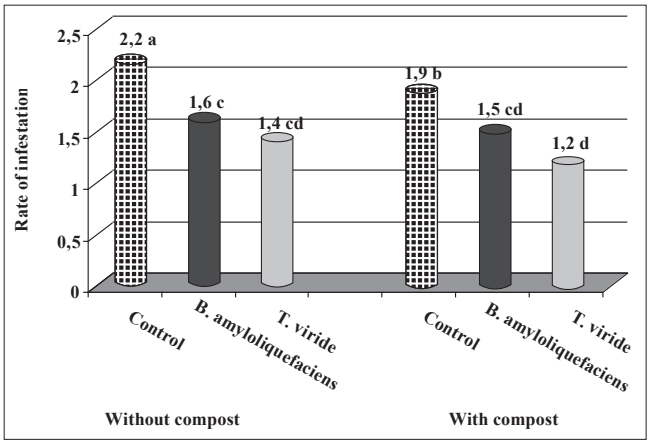

Fig. 1. Infestation rate by *Fusarium oxysporum* f. sp. *lycopersici* in tomatoes treated with *Bacillus amyloliquefaciens* strain A1 and *Trichoderma viride* strain T6

The variants where the plants, infested with *P. lycopersici* and treated with *B. amyloliquefaciens* and *T. viride* again demonstrate again higher biometrical indices (Table 6). There is no significant difference between the variants with and without compost. The differences between the control and other variants are insignificant. The values are higher for variants with compost than without compost. Plants with bacterial and fungous isolates had lower pathogen development. The highest infestation rate was in the control (Figure 2). These results confirm the depressing effect of the composts towards the soil pathogens, established by Sabet et al. (2013).

Root-knot nematodes

The results of three-way analysis of variance show that the variation in the rate of root-knot galling is significantly influenced by three main factors, as well as the interaction of A*B, B*C and A*B*C. Only the interaction of A*C did not have a significant effect. This means that in the individual years the variant of treatment would have a similar effect on nematode attacks (Table 7).

Significant differences in root-galling rates were recorded at the end of experiment. Variants with microbioagents had

Table 5
Influence of experimental factors on the infestation rate of *Pyrenochaeta lycopersici*

Source	Sum of Squares	df	Mean Square	η%	Sig.
Year (A)	0.03	1	0.03	0.09	
Medium (B)	0.83	1	0.83	2.24	
Variant (C)	10.87	2	5.43	29.24	***
A * B	0.03	1	0.03	0.09	
A * C	0.07	2	0.03	0.18	
B * C	0.87	2	0.43	2.33	
A * B * C	0.07	2	0.03	0.18	

η% - Power of influence in %, *p<0.05; ** p<0.01; *** p<0.001.

Table 6
Effect of *Bacillus amyloliquefaciens* strain A1 and *Trichoderma viride* strain T6 on plant growth of tomato variety Belle F_1 infected with *Pyrenochaeta lycopersici*

Variant	Shoot length, cm	Fresh shoot weight, g	Diameter of stem, mm	Root length, cm	Fresh root weight, g
Without compost					
Control	138.80±32.47 n.s.	205.50±57.65 b	7.65±0.79 c	32.54±13.00 n.s.	30.03±9.90 b
B. amyloliquefaciens	151.50±23.64 n.s.	233.75±66.80 ab	8.28±0.81 a	34.26±13.14 n.s.	33.11±10.01 b
T. viride	149.80±23.68 n.s.	232.00±54.73 ab	7.71±0.64 bc	35.34±13.27 n.s.	34.02±10.36 b
With compost					
Control	144.30±29.31 n.s.	228.00±60.18 ab	8.22±0.72 ab	34.26±15.59 n.s.	34.40±11.13 b
B. amyloliquefaciens	156.65±29.53 n.s.	254.50±44.33 a	8.59±1.08 a	37.91±13.71 n.s.	43.50±15.34 a
T. viride	156.85±29.09 n.s.	254.00±54.21 a	8.40±0.89 a	37.65±14.65 n.s.	35.55±8.44 b

a, b, c … n.s. – Duncan's multiple range test ($p < 0.05$).

significantly lower rates compared to the control with and without compost. The lowest root-galling rate (1.3) was established in the variant with compost and bacteria (Figure 3). This variant also had the highest average shoot length (138.80

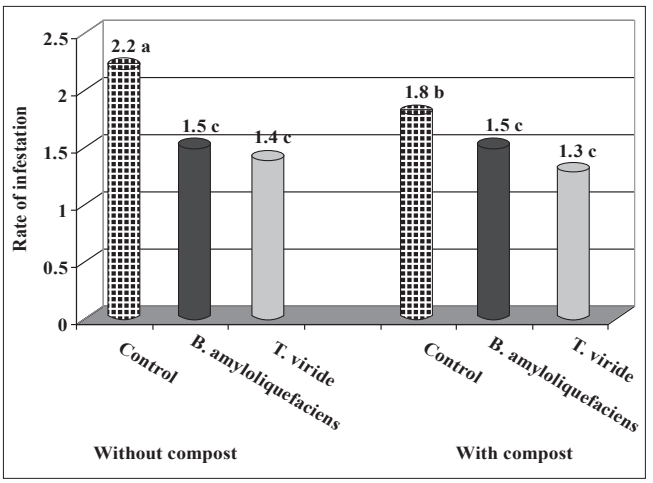

Fig. 2. Infestation rate by *Pyrenochaeta lycopersici* in tomatoes treated with *Bacillus amyloliquefaciens* strain A1 and *Trichoderma viride* strain T6

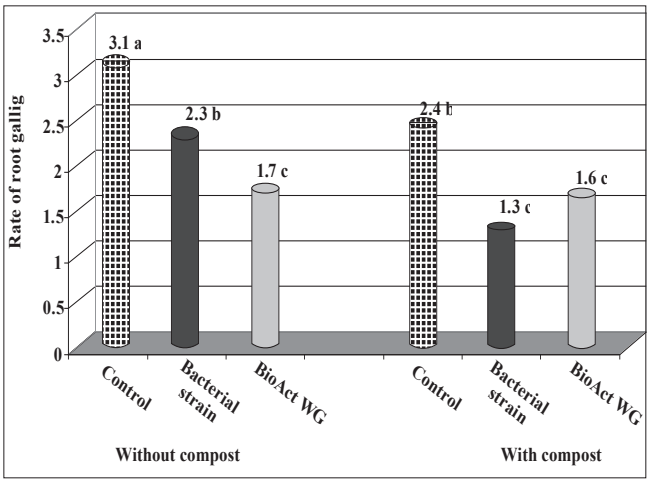

Fig. 3. Root-galling rate by *Meloidogyne* spp. in soil treated with bacterial strains (*Bacillus thuringiensis* strain Bt1 + *Bacillus amyloliquefaciens* strain 2/7A) and BioAct WG

Table 7
Influence of variation factors on tomato root-galling rates

Source	Sum of Squares	df	Mean Square	η%	Sig.
Year (A)	20.83	1	20.83	16.06	***
Medium (B)	10.80	1	10.80	8.33	***
Variant (C)	27.95	2	13.98	21.55	***
A * B	6.53	1	6.53	5.04	***
A * C	2.32	2	1.16	1.79	
B * C	4.85	2	2.42	3.74	**
A * B * C	3.62	2	1.81	2.79	*

η% - Power of influence in %, *p<0.05; ** p<0.01; *** p<0.001.

Table 8
Effect of bacterial strains (*Bacillus thuringiensis* strain Bt1 + *Bacillus amyloliquefaciens* strain 2/7A) and BioAct WG on growth of tomato variety Belle F_1

Variants	Shoot length, cm	Fresh shoot weight, g	Diameter of stem, mm	Root length, cm	Fresh root weight, g
Without compost					
Control	128.00±11.46 ab	228.10±18.14 d	8.64±1.21 ab	26.20±4.51 b	53.45±15.74 ab
Bacterial strain	129.30±14.97 ab	244.00±38.93 cd	8.72±1.06 ab	31.51±6.36 ab	57.22±31.39 a
BioAct WG	126.90±10.71 ab	231.30±30.71 cd	8.43±0.57 b	31.61±9.96 ab	35.63±10.20 d
With compost					
Control	102.80±8.85 b	261.00±21.32 bc	9.29±0.98 a	35.48±9.53 a	45.15±10.74 bd
Bacterial strain	138.80±15.23 a	307.40±44.48 b	8.89±0.83 ab	36.97±9.63 a	39.05±9.21 cd
BioAct WG	138.60±16.32 a	279.10±31.07 ab	8.65±0.96 ab	34.00±11.08 a	48.08±15.84 ac

a, b, c … – Duncan's multiple range test ($p < 0.05$).

cm), fresh shoot weight (307.40 g) and root length (36.97 cm) (Table 8).

Bacteria suppressed the development of root-knot nematodes and positively affected plant growth. Comparatively low root-galling rates were observed in variants with Bio-Act WG grown with and without compost, 1.6 and 1.7, respectively, compared to the control. All variants with compost had lower root galling rates than those grown without compost (Figure 3). This suggests that the use of compost is favourable to the development of beneficial microorganisms. Root weight was also greater: 39.05 g with compost and 35.63 g without compost, for trials with BioAct. This is probably due to lower root galling at the end of the experiment.

Conclusions

Microbioagents *Bacillus amyloliquefaciens* strain A1 and *Trichoderma viride* strain T6 were incorporated into soil infested with the soil pathogens *Fusarium oxysporum* f. sp. *lycopersici* and *Pyrenochaeta lycopersici*. The microbioagents reduced the effects of the pathogens and stimulated plant development. This effect was increased by adding compost to the soil. The lowest root-galling rate was for the variant with *Bacillus thuringiensis* strain Bt1 + *Bacillus amyloliquefaciens* strain 2/7A grown with compost.

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