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BIOLOGICAL CONTROL OF CORKY ROOT IN TOMATO

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SUMMARY

Corky root caused by *Pyrenochaeta lycopersici* (Schneider et Gerlach) is one of the most important soilborne fungal pathogens which develops in the soils, causing diseases in different crops. The research was carried out to evaluate the effectiveness of the biological control of corky root on tomato. Biological control was performed by using *Trichoderma viride* Pers. 18/17 SS, *Streptomyces* spp. AtB42 and *Bacillus subtilis* M51 PI.

In laboratory, the effectiveness of *T. viride* 18/17 SS, *Streptomyces* spp. AtB42 and *B. subtilis* M51 PI to control *P. lycopersici* were studied. In greenhouse, the research was carried out comparing the following treatments: 1) untreated control; 2) *T. viride* 18/17 SS; 3) *Streptomyces* spp. AtB42; 4) *B. subtilis* M51 PI. Roots of plants of tomato H3028 Hazera were treated with the antagonist suspensions just prior of transplant. Treatments were repeated about 2 months after, with the same suspensions sprayed on the soil to the plant collar. In dual culture, the inhibition of *P. lycopersici* ranged up to 81.2% (caused from *T. viride* 18/17 SS), 75.6% (from *Streptomyces* spp. AtB42) and 66.8 % (from *B. subtilis* M51 PI).

In greenhouse trials, with regard to corky root symptoms, all treated plots showed significative differences compared to untreated. *T. viride* gave the better results followed by *Streptomyces* spp. and then by *B. subtilis*. The fungus antagonist showed good root surface competence such as demonstrated its persistence in the rizhosphere of tomato plants up to 2 months before.

INTRODUCTION

Corky root caused by *Pyrenochaeta lycopersici* (Schneider et Gerlach) is one of the most important soilborne fungal pathogens which develops in the soils, causing diseases in many crops. Therefore the research was carried out to evaluate the effectiveness of the biological control of corky root on tomato. Biological control was performed by using some strains of *Trichoderma viride*, *Streptomyces* spp. and *Bacillus subtilis*. *T. viride* is a filamentous soil fungus known as an effective biocontrol agent of a wide range of air borne and soil borne pathogens. These fungi grow toward hyphae of other fungi, coil with them in a lectin-mediated reaction, and degrade cell walls of the target fungi by secretion of different lytic enzymes. *Streptomyces* spp. are gram-positive, filamentous, soilborne bacteria that also occur in the phylloplane. Much *Streptomyces* strains are disease suppressive and employed in the biological control of some phytopathogenic fungi. *B. subtilis* subsp. *subtilis* is a gram-positive harmless bacterium able to produce secretion of volatile and diffusible metabolites, but not fungal cell wall hydrolysing enzymes, causing *in vitro* inhibition of some root fungi. According to present and future regulations on the use of chemical fungicides and considering that treatments must avoids environmental pollution, the main object of this research was to find alternative strategies by using biocontrol agents.

MATERIALS AND METHODS

In laboratory, the effectiveness of strains *T. viride*, *Streptomyces* spp. and *B. subtilis* to control *P. lycopersici* were studied. Antagonist and pathogen combinations were examined on PDA in 9.2 cm Petri dish with 4 replications. For dual culture of *T. viride*, a mycelial disc (0.9 cm diam), obtained from the edge of 15-day-old cultures of *P. lycopersici*, was placed on a PDA plate (3 cm from the centre). Then, a 0.9 cm diam mycelial disc, obtained from the edge of a 4 day-old culture of *T. viride*, was placed 3 cm from the plate centre, opposite to the pathogen inoculum. The two inocula (pathogen and antagonist) were 6 cm distant. The plates were incubated at 28 °C, and daily measurements of the radial growth were taken. In the control a sterile agar disc (0.9 cm diam) was placed in the dish instead of *T. viride*. For dual culture of bacterial antagonists, half of the agar surface in dishes was smeared with a suspension of one bacterial isolate in sterile distilled water by using a sterile round wire of platinum. After incubation at 28 ºC in the dark for 24 h for *B. subtilis* and 48 h for *Streptomyces* spp., a plug 0.9 cm diameter cut from the leading edge of 15 day-old culture of *P. lycopersici* on PDA medium was placed on the other half of the dish at 0.5 cm from the border of the plate. For control, dishes were inoculated with pathogen alone, without antagonists. Dishes were incubated at 25 ºC in the dark for 18 days. Radial growth of *P. lycopersici* colony was daily measured in normal direction of the antagonist (*Streptomyces* spp. or *B. subtilis*).

The inhibitory halo was detected and measured at the end of all the *in vitro* experiments, after 18 days from the inoculation. All the experiments in laboratory were replicated four times.

Inhibition of *P. lycopersici* by the antagonists was calculated in relation to the growth of the control, by using a formula that measures the percentage of the inhibition of the radial mycelial growth.

In greenhouse, the research was carried out at the farm of Vegetable Crop Research Center of Pontecagnano (Salerno). The following treatments were compared by a randomized complete block experiment design, replicated four times, by using tomato plants of "H3028 Hazera F1": 1) untreated control; 2) *T. viride* 18/17 SS; 3) *Streptomyces* spp. AtB42; 4) *B. subtilis* M51 PI. Treatments were repeated about 2 months after, with the same suspensions sprayed on the soil to the plant collar. Each plot was 2.4 m x 1.2 m (surface 7.2 m2), with 9 plants (density 12.500 plants/Ha). Plants were transplanted on 14 November in soil with a history of corky root caused by *P. lycopersici*. At the same day, before the transplant, the roots of tomato plantlets were immersed in sterile water (untreated control), or in suspension of *T. viride* 18/17 SS at 50.000 conidia/mL, or in cell suspension of *Streptomyces* spp. AtB42 at 3 x 106 CFU/mL, or in cell suspension of *B. subtilis* M51 at about 2 x 109 CFU/mL. Treatments were repeated on 23 January 2001, with the same suspension concentrations of the first treatment. In this last case, the suspension of conidia or cells was inoculated on the soil to the plant collar, using 100 mL/plant. After each treatment the soil was soft irrigated by drop irrigation. Phytopathological observations were carried out on 3 plants/plot, monthly and starting on 4 April. Plant roots were randomly collected, water-washed and the symptom severity was rated visually according to the following scale: 0 = healthy roots; 1 = 1-9% of brown and sloughing root surface; 2 = 10-24%; 3 = 25-49%; 4 = over 50-75%, 5 = over 75%.

The soil microbial analysis were carried out. Samples were taken randomly from the rizhosphere of tomato plants of the compared treatments. Ten grams of mix (soil and small roots) was added to sterile distilled water (1:9, wt/vol) and shaken for 10 min on a rotary shaker, serial dilutions were made, and one aliquot was inoculated to measure the microbial population on TSA medium for *Bacillus* spp., or SCA medium for *Streptomyces* spp., or PDA plus Bengala pink (for fungi). After incubating plates at 28 °C for 3 days, the colonies were counted and colony forming units (CFU) were calculated and expressed such CFU/mg of soil. The experiment was three times replicated.

Meteorological data were collected in open field from the Agrometeorological Station 00220 of Pontecagnano (Salerno) 29 m alt, 4037' lat, 1452' lon (SIAN, CRA, UCEA), during the experiments.

CONCLUSIONS

Laboratory trials showed the antagonistic ability of *T. viride* 18/17 SS, *Streptomyces* spp. AtB42 and *B. subtilis* M51 PI against *P. lycopersici*. For *T. viride*, the antagonism is based on quick occupation of the vital space, on direct competition for the nutritive elements and on a mycoparasitism activity observed in the contact points of the colonies in dual culture (coilings, strings and short branches). The non-appearance of the inhibition halo proves the absence of antibiosis based on the synthesis of chemical substances. For *Streptomyces* spp. and *B. subtilis* the antagonism is based on their secretion of antibiotics, antifungal metabolites, and enzymes demolishing the organic substances such as chitin that is the main component of the cellular wall of the fungi.

In greenhouse experiments the antagonists increase their population density in the soil and showed a good control of the corky root disease. The favorable agrometeorological values and the conditions of good soil (neutral pH, absence of salinity, no excess of water obtained by drop irrigation) contribute to improve the antagonist populations and the biocontrol against corky root.

RESULTS AND DISCUSSION

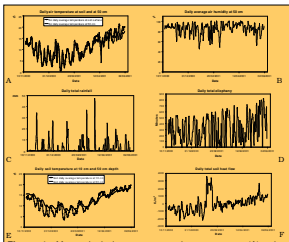


Figure 1. Meteorological parameters: air temperature (A), air humidity (B), rainfall (C), eliophany (D), soil temperature (E) and soil heat flow (F)

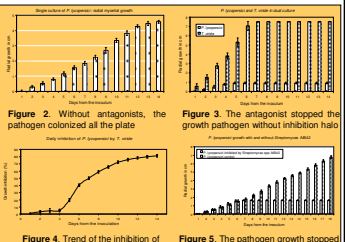


Figure 2. Without antagonists, the pathogen colonized all the plate

Figure 3. The antagonist stopped the growth pathogen without inhibition halo

Figure 4. Trend of the inhibition of *P. lycopersici* caused from *T. viride*

Figure 5. The pathogen growth stopped after 7 days when the radial growth was 1.7 cm. The inhibitory halo was 1.8 cm

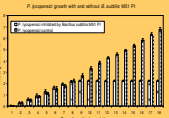


Figure 6. The *P. lycopersici* growth stopped after 9 days, when the radial growth was 2.3 cm. The inhibitory halo was 1.0 cm

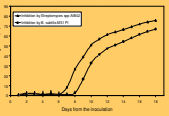


Figure 7. *Streptomyces* spp. AtB42 showed the best behaviour in controlling *P. lycopersici* compared with *B. subtilis* M51

Table 1. Values of the McKinney index (%) of the compared treatments in the control of corky root, during the growth of tomato plants in greenhouse (*)

Treatments	Days from transplant		
	141	171	199
Control (untreated)	40.0 A	53.3 A	66.7 A
T. viride	3.3 C	13.3 C	23.3 D
Streptomyces spp.	10.0 B	16.7 C	33.7 C
B. subtilis	16.7 B	30.0 B	43.3 B

(*) Values separation in columns by Duncan's multiple range test (P≤0.01).

Table 2. Microbial population (CFU/mg of soil) found in the rhizosphere of tomato plants for the compared treatments (*)

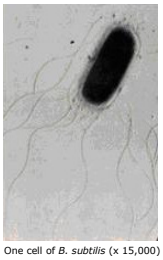
Treatments	Date of the soil sampling: days from transplant								
	20			69			171		
	Bacillus CFU	*Streptomyces* CFU	Fungal CFU	*Bacillus* CFU	*Streptomyces* CFU	Fungal CFU	*Bacillus* CFU	*Streptomyces* CFU	Fungal CFU
Control	157.0 B	4.0 B	5.7 B	136.7 A	1.3 A	4.3 B	211.0 B	14.0 B	101.0 B
T. viride	148.3 B	4.7 B	94.3 A	146.0 A	1.0 A	58.7 A	197.7 B	11.3 B	167.3 A
Streptomyces	172.3 B	55.7 A	4.0 B	154.0 A	12.7 A	5.7 B	198.3 B	84.7 A	88.7 B
B. subtilis	436.7 A	2.7 B	8.7 B	128.3 A	2.3 A	6.0 B	439.7 A	9.0 B	111.3 B

(*) Values separation in columns by Duncan's multiple range test (P≤0.01).

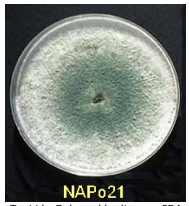
Meteorological data are reported in figure 1. The single culture of *P. lycopersici* showed that the radial growth was 4.6 cm occupying all the available space of the dishes in 14 days (Figure 2). In dual culture of *P. lycopersici* and *T. viride* radial growth of the pathogen fungus did not overgrow 0.9 cm. In fact, growing quickly, *T. viride* reached the periphery of the *P. lycopersici* colony, preventing the development of the fungus not over 0.9 cm. The radius of *T. viride* was over 7.5 cm, after 7 days from the inoculation (Figure 3). The inhibition of *P. lycopersici* caused from *T. viride* ranged up to 81.2% (Figure 4). In dual culture of *P. lycopersici* and *Streptomyces* spp. AtB42, the maximum radial growth of the pathogen was 1.7 cm. This maximum development happened in 7 days and was stopped by the antagonist. After 18 days, *P. lycopersici* alone cultured (control) reached the average development of 6.8 cm, occupying the space available (figure 5). The inhibition halo was 1.8 cm. *P. lycopersici* co-cultured with *B. subtilis* M51 showed the maximum development after 8 days when the radial growth ranged up to 2.3 cm. (figure 6). The inhibitory halo of 1.0 cm was observed, suggesting the presence of fungistatic metabolites secreted by the *B. subtilis*. In dual culture, the daily inhibition of *P. lycopersici* by *Streptomyces* spp. ranged up to 75.6% while that caused from *B. subtilis* ranged up to 66.8 %. The inhibition average difference of *P. lycopersici* induced by the two antagonists was 24.8% (figure 7).

In greenhouse trials all treated plots showed significant differences from untreated. *T. viride* gave the better results (table 1). The microbial population density of the soil gave the lowest values for control at the observations that were carried out 20 days after first soil inoculation and at the end of the experiment. Two months after the first inoculation and before the second antagonist treatment, only *T. viride* showed significant difference from untreated control. *T. viride* showed good persistence in the rizhosphere of tomato plants up to 69 days after its root treatment. After the second soil treatment with the antagonists, the microbial population density increased until 440 CFU (*B. subtilis*) 85 CFU (*Streptomyces* spp.) and 167 CFU (*T. viride*) at the observation of the soil sampling of 171 days from the transplant (table 2).

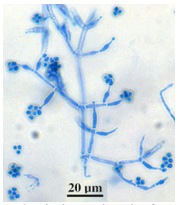
The increase of the microbial population was due, besides the integration of the antagonists according to the soil management program, also to the improvement of the agrometeorological parameters (figure 1).



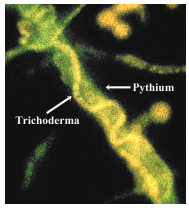
One cell of *B. subtilis* (x 15,000)



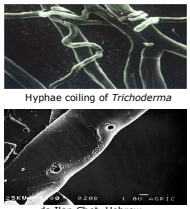
T. viride 7-days-old culture on PDA



Conidiophora and conidia of *Trichoderma*

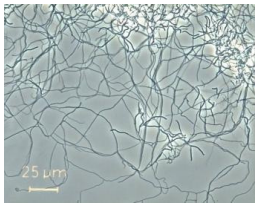


from Hubbard *et al.*, 1983. *Phytopathology*, 73:655-9

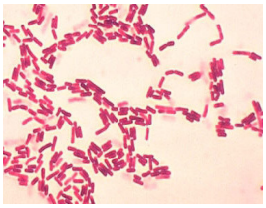


Hyphae coiling of *Trichoderma*

da Ilan Chet, Hebrew University of Jerusalem



Vegetative filaments of *Streptomyces* spp.



Cells of *B. subtilis*