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Biocontrol of Sugarbeet Pathogen *Fusarium solani* (Mart.) Sacc. by *Streptomyces aureofaciens*

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Abstract: The potential of microbial antagonism was explored in the control of sugarbeet *Fusarium* disease. The *in vitro* studies showed that a 70% concentration of the culture filtrate of *S. aureofaciens* significantly inhibited the spore germination, mycelial growth and sporulation of *Fusarium solani*. The *in vivo* studies involved different treatments. Seed coating treatment was the most effective in controlling *F. solani* at all cultivation periods in all the three-sugarbeet cultivars Raspoly, TOP and Tribel. The former cultivar showed the highest growth response compared with the other two cultivars. Soil pre-inoculation was less effective whereas seed-soaking treatment was the least effective in this respect.

Key words: Biocontrol, *Streptomyces, Fusarium*-diseases, sugarbeet, pathogenicity

Introduction

The use of the antagonistic properties of microorganisms, including different *Streptomyces* spp. in the biological control of many plant diseases has been the subject of many studies (Singh and Mehrotra, 1980; Turhan, 1981; Rothrock and Gottlieb, 1984; Lahdenperä, 1987; Martin and Hancock, 1987; Hayashida *et al.*, 1989). In all these studies, different methods were used for the application of the antagonistic component.

Streptomyces griseoviridis has been reported as an antagonist to the plant pathogens *Alternaria brassicicola, Botrytis cinerea, Fusarium avenaceum, F. culmorum, F. oxysporum* f.sp. *dianthi, Pythium debaryanum, Phomopsis sclerotioides, Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Tahvonen, 1982a, b; Lahdenperä, 1987; Tahvonen and Avikainen, 1987; Tahvonen and Lahdenperä, 1988). *In vitro* tests revealed that *S. griseoviridis* suppressed the growth of these fungi, the *in vivo* treatment of lettuce seedlings with a spore suspension from *Streptomyces* isolate significantly reduced yield losses caused by *B. cinerea*, without any effect on those caused by *R. solani*. The *in vitro* antimicrobial effect against *R. solani* was weaker than its effect against other tested pathogens (Tahvonen, 1982a). *In vivo* it controlled foot rot and damping-off caused by *A. brassicicola, R. solani* on cauliflower and *P. debaryanum* on sugarbeet. It also reduced the mortality of barley sprouts and foot rot caused by *F. culmorum* (Tahvonen, 1982b; Tahvonen and Avikainen, 1987).

Seed dusting with *Streptomyces* spp. prevented or reduced foot rot and damping-off of crucifers caused by *Alternaria brassicicola* and *Rhizoctonia solani*. Spraying the substrate with a suspension of *Streptomyces* reduced root diseases of cucumbers caused by *Pythium* spp. and in many cases successfully prevented carnation wilt due to *Fusarium oxysporum*. The microbe was most effective in peat soil, although good results were obtained in fine and clay soils also (Tahvonen, 1988).

In Egypt, several studies examined the possible utilization of antagonistic microorganisms in the biological control of phytopathogenic microorganisms (El-Abyad *et al.*, 1993 a, b, 1996; El-Shanshoury *et al.*, 1994, 1996; Moussa, 1999). Sirry *et al.* (1981) isolated *Streptomyces* spp. from the rhizosphere of sesame that were antagonistic against the root-rot pathogen *Fusarium oxysporum* and *Sclerotium bataticola*.

In this work we studied the biological control of *Fusarium*-disease of sugarbeet and deduced the best application method of the antagonistic *Streptomyces aureofaciens* as an alternative method to the application of fungicide.

Materials and Methods

Microorganisms, culture conditions and host plant: *Fusarium solani* (Mart.) Sacc. was isolated from diseased sugarbeet roots (El-Abyad *et al.*, 1988), described by El-Abyad and Abu-Taleb (1990) and maintained on the medium described by Johnson and Curl (1972).

Streptomyces aureofaciens shown to be potent antagonists to some pathogenic fungi (Moussa, 1999), was maintained on starch nitrate slants (Waksman and Lechevalier, 1961).

Seeds of sugarbeet (*Beta vulgaris* L.) cultivars 'Raspoly', 'TOP', and 'Tribel' were obtained from the North Delta Sugar Company, Egypt.

Effect of filtrates of *S. aureofaciens* on growth activities of *F. solani in vitro:* *S. aureofaciens* was grown on starch nitrate agar for 10 days at 29°C on plates. The sporulating aerial hyphae were removed then 10ml sterile distilled water was added to the substrate mycelium. The mixture was homogenized and filtered through Whatman No. 1 paper and the filtrate was filter-sterilized through 0.45 μm filter. Different concentrations of the filtrate (30, 50, 70 and 90%, v/v) were prepared with sterile starch nitrate broth.

Germination of fungal spores and lengths of germ tubes were studied employing microscopic slides according to the methods described by El-Abyad *et al.* (1983). Radial growth was determined by mixing aliquots of filtrates aseptically, with sarcina agar medium to give concentrations ranging from 30-90% (v/v), and poured in petri dishes. *F. solani* was grown on tap water agar (1.5%, w/v) at 28°C for 7 days. Disks (5 mm) were cut from the margins of colonies and transferred to the sarcina agar plates amended with filtrate for a period of 7 days at 28°C. Control without filtrate were prepared.

Sporulation was studied by inoculating plates containing sarcina-amended agar medium with a 5-mm disk of tap water agar bearing fungal mycelium, and subsequently incubating them at 28°C for 10 days. The density of spores/ml was counted using a hemocytometer as described by El-Abyad *et al.* (1983). Control sarcina agar plates without filtrate were prepared.

Preparation of inocula for biological control treatments: For seed-coating treatment, *S. aureofaciens* was grown at 28°C for 7 days on starch nitrate agar. The hyphae and spores of three plates were suspended in 15-ml sterile distilled water in which seeds were coated as described by Singh and Mehrotra (1980). Both pathogen inoculation and treated seeds were done simultaneously. Treated seeds were air-dried for 30 min before sowing.

For soil pre-inoculation treatment, the *Streptomyces* strain was grown on starch nitrate broth on a rotatory shaker (200-rpm) at 28°C, the hyphae were harvested by centrifugation at 3000 *g* for 20 min and the pellets resuspended in 10 ml of sterile distilled water. The strain was inoculated into the soil 7 days prior to the inoculation of pathogen and seed sowing at a rate of 0.6g Kg^{-1} soil.

The treatment with seeds soaked in the filtrate of antagonistic *Streptomyces* sp. was done with filtrates or distilled water as control for 30 min prior to the sowing and infestation of soil. Control treatments included seeds soaked in sterile distilled water.

F. solani was grown on sarcina agar medium for 7 days. Agar disks (5-mm) bearing mycelium were inoculated into sarcina broth in Erlenmeyer flasks and incubated at 28°C for 3 days. The mycelia were harvested on Whatman No. 1 paper. These were weighed, resuspended in sterile distilled water and blended for 30 sec. *F. solani* was inoculated into the soil at a rate of 0.2g Kg^{-1} soil (Rothrock and Gottlieb, 1984).

The pots were kept in the greenhouse for 60 days. After 2, 4, 6 and 8 weeks from planting, the plants were removed, washed with tap water and the following measurements were made: % emergence, % infection, root depth, root dry weight, number of leaves, shoot dry weight of plants.

Statistics: The data recorded, here in, are the means of at least 3 replicates and the results were analyzed according to Sokal and Rohlf (1981).

Results

Effect of culture filtrate of *S. aureofaciens* on growth activities of *F. solani in vitro:* The percentage germination and average germ-tube length of spores of *F. solani* decreased significantly by raising the concentration of culture filtrate of *S. aureofaciens*. However, 70% filtrate concentration was arrested (Table 1). The number of *F. solani* spores significantly decreased with increasing filtrate concentration of *S. aureofaciens*.

Results in Table 1 show that different concentrations of *S. aureofaciens* filtrate significantly inhibited growth of *F. solani* at all incubation periods. The extent of inhibition increased with increased concentration and/or incubation period. Similarly the dry mass yield of the fungal pathogen significantly decreased with increasing the filtrate concentration.

Biological control of sugarbeet fungal disease: Under different infestation treatments, the percentage emergence of Raspoly and Tribel sugarbeet seedlings significantly decreased whereas in TOP seedlings significantly increased as compared with non-infested control (Table 2). Soil pre-inoculation and seed coating treatments significantly raised whereas seed soaking treatment non-significantly affected emergence compared with the infested soil. The infection percentage significantly increased in infested control with progress of time, but decreased under soil pre-inoculation or seed soaking treatments, compared with infested soil. The infection percent also increased with the progress of cultivation period. The infection percentage was nil in seed coating treatment in all three sugarbeet cultivars.

Infestation of soil with *F. solani* alone, suppressed the growth parameters of sugarbeet cultivars Raspoly, TOP and Tribel, while application of different treatments with *S. aureofaciens* nullified or even reversed the inhibitory effects of the pathogen (Table 3).

Table 1: Effect of different concentrations of cell-free extract of *Streptomyces aureofaciens* cultures on the percentage germination (G%) and average length of germ-tubes (Gt) after 16 h incubation period as well as the number of spores, on the dry weight yields after 10 days and on the radial growth after various incubation periods of *Fusarium solani* at 28°C

Filtrate conc. (%)		G (%)	Gt (μm)	No. of spores (x 10^5 ml^{-1})	Dry wt. (mg)	Radial growth (mm)		
						2 day	4 day	6 day
Control		80.3	110.2	142.6	1107.7	23.5	55.6	85.5
30		66.7**	85.0**	104.8**	880.3**	20.2**	24.5**	35.5**
50		35.3**	32.0**	77.6**	220.7**	13.2**	15.3**	20.6**
70		0.0**	0.0**	39.2**	160.3**	8.0**	10.5**	15.8**
90		0.0**	0.0**	5.6**	90.7**	5.0**	5.0**	5.5**
	5%	3.9	3.5	4.9	9.3	1.1	2.0	2.4
LSD	1%	5.7	5.4	6.5	16.6	2.6	2.3	4.8

Note: **, highly significant at 1% LSD; *, significant at 5% LSD related to control.

Table 2: Effect of different treatments with *S. aureofaciens* on the control of sugarbeet pathogen *F. solani* on sugarbeet cultivars Raspoly, TOP and Tribel during 60 days post sowing

Treatment	Emergence (%)	Infection (%)			
		15 day	30 day	45 day	60 day
Raspoly					
Non-infested control	85.0	0.0	0.0	0.0	0.0
Infested control	73.5**	3.3**	19.4**	39.3**	70.5**
Soil pre-inoculation	80.0**††	0.0**††	0.0††	0.0††	12.5**††
Seed coating	80.0**††	0.0**††	0.0††	0.0††	0.0††
Seed soaking	71.7**	0.0**††	6.4**††	12.1**††	41.9**††
LSD 1%	3.3	0.0	6.4	6.1	1.9
5%	2.4	0.0	4.5	4.3	1.4
Top					
Non-infested control	76.7	0.0	0.0	0.0	0.0
Infested control	66.7**	5.6**	31.7**	65.8**	82.6**
Soil pre-inoculation	78.3††	0.0**††	0.0††	0.0††	19.2**††
Seed coating	78.3††	0.0**††	0.0††	0.0††	0.0††
Seed soaking	71.7**††	0.0**††	6.4**††	8.8**††	55.8**††
LSD 1%	3.3	0.0	6.4	0.5	2.7
5%	2.4	0.0	4.5	0.4	1.9
Tribel					
Non-infested control	80.0	0.0	0.0	0.0	0.0
Infested control	66.7**	13.4**	35.0**	48.9**	77.5**
Soil pre-inoculation	73.3**††	0.0**††	0.0††	0.0**††	20.5**††
Seed coating	78.3††	0.0**††	0.0††	0.0**††	0.0††
Seed soaking	73.3**††	0.0**††	6.4**††	9.1**††	52.2**††
LSD 1%	3.3	0.0	6.4	0.0	2.2
5%	2.4	0.0	4.5	0.0	1.6

Note: **, highly significant at 1%; *, significant at 5% related to non-infested control.
††, Highly significant at 1%; †, significant at 5% related to infested control.

Table 3: Effect of different treatments with *S. aureofaciens* on the growth parameters of the sugarbeet cultivars Raspoly, TOP and Tribel during 60 days post sowing

Treatment	Root length (mm)				Dry wt. (g)			
	15	30	45	60	15	30	45	60
Raspoly								
Non-infested control	30.0	45.0	80.0	100.0	2.9	4.5	7.2	10.8
Infested control	25.0**	42.0	63.0**	83.0**	2.3**	4.2**	6.7**	9.4**
Soil pre-inoculation	29.0††	48.0††	77.0††	101.0††	2.6*†	4.4**††	7.9*††	10.9*††
Seed coating	33.0**††	55.0**††	86.0*††	114.0**††	3.0††	5.1**††	8.8**††	12.1**††
Seed soaking	27.0**††	45.0	72.0**††	95.00††	2.6*†	4.2**††	7.1*†	9.8**††
LSD 5%	1.4	3.4	4.8	5.8	0.1	0.08	0.1	0.1
1%	2.0	4.8	6.8	8.2	0.2	0.1	0.2	0.2
Top								
Non-infested control	20.0	40.0	80.0	102.0	1.8	4.0	6.1	8.5
Infested control	10.0**	25.0**	59.0**	74.0**	1.0**	3.6**	5.4**	7.8**
Soil pre-inoculation	8.0**††	47.0**††	79.0††	99.0††	1.0**††	3.8**††	6.4**††	8.6*††
Seed coating	16.0**††	50.0**††	88.0*††	110.0**††	1.7**††	4.6**††	8.6**††	11.3**††
Seed soaking	10.0**	39.0††	67.0**††	83.0**††	1.0**††	3.2**††	5.8**††	7.8**
LSD 5%	0.8	1.4	6.2	4.1	0.0	0.09	0.04	0.1
1%	1.2	2.0	8.8	5.8	0.0	0.1	0.06	0.2
Tribel								
Non-infested control	23.0	43.0	67.0	90.0	2.7	5.3	8.4	11.8
Infested control	21.0**	38.0**	63.0*	81.0**	2.0**	4.1**	6.0**	8.6**
Soil pre-inoculation	25.0**††	45.0††	73.0**††	97.0**††	2.3**††	5.7**††	9.4**††	11.3*††
Seed coating	30.0**††	51.0**††	82.0**††	108.0**††	3.4**††	7.9**††	12.0**††	16.8**††
Seed soaking	24.0††	43.0††	70.0*††	93.0††	2.3**††	4.3**†	7.5**††	9.8**††
LSD 5%	1.4	2.9	2.2	4.0	0.1	0.2	0.3	0.4
1%	2.0	4.1	3.1	5.7	0.2	0.4	0.4	0.6

Note: **, highly significant at 1% LSD; *, significant at 5% LSD related to non-infested control.
††, Highly significant at 1% LSD; †, significant at 5% LSD related to infested control.

In the three sugarbeet cultivars, the antagonistic *S. aureofaciens* when applied as seed coating is the best for controlling fungal pathogen as well as increasing the growth of the sugarbeet cultivars.

Discussion

The *in vitro* studies showed that 70 % of the culture filtrate of *Streptomyces aureofaciens* completely inhibited the spore germination and mycelial growth, but significantly decreased the sporulation of *Fusarium solani*. Many microorganisms are capable of producing antibiotics following stimulation by the appropriate substrates (Takeuchi *et al.*, 1988). Similar studies were done with different microorganisms antagonistic against various pathogens *Alternaria solani*, *Aspergillus niger*, *Curvularia pallescens* and *Helminthosporium oryzae* inhibited by the water soluble antibiotics produced by *Streptomyces galbus* (Paul and Banerjee, 1986).

The results indicated that the control of pathogen and growth of sugarbeet cultivars differed according to the treatment, the cultivar and the length of cultivation period. After 15 days of treatment, no infection symptoms have been detected in all cultivars. The growth parameters of the three cultivars (Raspoly, TOP, Tribel) have been significantly increased by different *Streptomyces* treatments. After 30, 45 and 60 days, the best treatment for controlling the pathogen by *S. aureofaciens* was the seed coating treatment. This may be due to the spores of the strain in contact with seed of sugarbeet, which continuously supplied the seed with the antimicrobial compound (s). Similar results were obtained by other workers in the control of maize root rot (Singh and Mehrotra, 1980); *Fusarium* root rot of faba bean (Yehia *et al.*, 1982); damping-off of sugarbeet (Martin and Hancock, 1987); tomato wilt (El-Abyad *et al.*, 1993a). In all these studies and in our work, the seed coating treatment improved plant growth may be due to the growth regulators produced by the antagonist together with their continuous supply to the developing plants as a result of the intimate contact between the seeds and the antagonist.

Inoculation of soil with the antagonist 7 days prior to sowing was less effective in controlling the pathogen than the seed coating treatment. No symptoms were observed in soil pre-inoculation treatment with *S. aureofaciens* at 15, 30 and 45 days post sowing. On the other hand, the increase in the percentage infection by *F. solani* at the late stage of cultivation in the soil pre-inoculation treatment may indicate that *F. solani* was more tolerant to the environmental conditions. This may be also due to the decline of the antagonistic strain population resulting in a decreased production of antimicrobial substances. At the early stages of growth, the decrease of infection may be due to the high population of *S. aureofaciens*. Similar studies were applied for the control of root rot of pea seedlings (Rothrock and Gottlieb, 1984); damping-off of cauliflower (Kundu and Nandi, 1993).

This investigation also showed that the seed soaking treatment was least effective in controlling *F. solani* compared with other treatments. This may be attributed to the decreased absorptive capacity of sugarbeet seeds for the antagonistic compounds due to their hard coat and hence low-level accumulation in the germinating seeds. Our findings are confirmed, to some extent, in controlling wilt of soybean and french bean (Khalid, 1987); root disease of cucumbers (Tahvonen, 1988); damping-off of sugarbeet (Rath and Wolf, 1992); wilt of tomato (El-Abyad *et al.*, 1993a).

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