

BIOLOGICAL CONTROL OF SOME SOIL BORNE PATHOGENS AFFECTING TOMATO AND PEPPER PLANTS

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ABSTRACT

Trichoderma harzianum, *Gliocladium virens*, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Actinomyce* isolate which proved to have substantial effect against the soil borne pathogens causing wilt and root-rot diseases of tomato and pepper plants. Under greenhouse conditions results showed that significant reduction in post-emergence damping-off and disease index of tomato plants caused by *Fusarium oxysporum* f. sp. *lycopersici* and *Rhizoctonia solani* were achieved by the application of the tested selected antagonists. Similar levels of protection of pepper plants against *Fusarium solani*, *R. solani* and *Pythium aphanidermatum* were achieved by the selected tested antagonists. However, *T. harzianum* and *G. virens* were the best antagonists among the tested bioagents.

INTRODUCTION

Non-fungicidal applications for plant fungal diseases is one of the major objectives of the plant pathologists all over the world for avoiding hazardous of using chemicals.

Biological control of plant diseases has gained the increasing interest in the last two decades (Lumsden *et al.*, 1992; Benhamou and Chet, 1996 and Khalifa, 1997).

Many bacterial and fungal antagonists were found to have substantial effect against many soil-borne pathogens (Papavizas, 1985; Park *et al.*, 1991; Shahida *et al.*, 1991; Phae *et al.*, 1992; Saracchi *et al.*, 1992; Wolk and Sorkar, 1994; Benhamou *et al.*, 1996 and Khalifa and Liddell, 1996).

The target of the present work is to study the effect of certain biocontrol agents on some soil borne pathogens causing wilt, root-rot and damping-off of both tomato and pepper plants.

MATERIALS AND METHODS

Serious soil-borne pathogens i.e., *Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani*, *Fusarium solani* and *Pythium aphanidermatum* which were isolated from diseased tomato and pepper

plants collected from different Delta Governorates at different stages of growth during 1995-1996 seasons by Hammoud (2000).

The biocontrol agents used in the present work, i.e., *Bacillus subtilis*, *Pseudomonas fluorescens*, *Actinomyces sp.*, *Trichoderma harzianum* and *Gliocladium virens* were originally isolated from healthy root and rhizosphere of tomato and pepper plants by El-Kazzaz et al. (2001).

I. Greenhouse experiments:

The effect of the most effective bacterial isolates for controlling root-rot disease incidence of tomato and pepper seedlings was studied in non-sterilized soil in pots.

Preparation of bacterial inoculum and infesting soil:

Inocula of the antagonistic isolates were prepared by growing them on nutrient broth media for *B. subtilis* or on King's broth media for *P. fluorescens* or starch nitrate medium for *Actinomyces sp.* in conical flasks (500 ml) at 28°C for 5 and 10 days, respectively using shaking incubator (100 rpm). Cell suspension was diluted and adjusted to 10^8 CFU/ml of *B. subtilis* and *P. fluorescens* or 10^7 propagules of *Actinomyces sp.* Soil was infested with each of the antagonistic bacterial isolates or *Actinomyces sp.* at time of planting with the specific concentration.

The different antagonistic fungi were grown on wheat bran medium (Elad et al., 1980). Autoclaved bottles contain 200 g of the medium, were inoculated with 6 mm diameter agar disks of each of the tested antagonistic fungal isolates and incubated for 15 days at 28°C with each of the biotic fungal isolates on the day of planting at the rate of 3% of soil weight for *T. harzianum* and 2% of soil weight for *G. virens*. Infested soil treated with benomyle was used for comparison, while untreated soil was used as a check. All *in-vivo* experiments were conducted inside the greenhouse of Dept. of Agric., Botany, Faculty of Agriculture, Kafr El-Sheikh, Tanta University.

Statistical analysis :

Complete randomized design was applied to laboratory and greenhouse experiments. Data were subjected to analysis of variance according to Duncan (1955) using the computer program (IRRISTAT).

RESULTS AND DISCUSSION

The *in vivo* experiments:

All the tested biocontrol agents i.e., *T. harzianum*, *G. virens*, *P. fluorescens*, *B. subtilis* and an *Actinomyces sp.* significantly reduced damping-off, wilt and root-rot of tomato caused by *F. oxysporum* f. sp.

lycopersici and *R. solani* (Tables 1 and 2) or pepper plants caused by *F. solani*, *R. solani* and *P. aphanidermatum* (Tables 3, 4 and 5) under greenhouse conditions during 1997 and 1998 growing seasons. It is clear from the obtained results that the tested antagonists had significant effect in protecting tomato and pepper plants against each of the studied pathogens. It was found by many investigators that among 20 genera of bacteria, different *Bacillus spp.*, *Pseudomonas spp.* and *Streptomyces spp.* were widely used as biocontrol agents of soil-borne pathogens (Cook and Baker, 1983 and Yuen *et al.*, 1985). However, *B. subtilis* and *P. fluorescens* in particular appear to be the most effective biocontrol agents for its ability to control some soil-borne fungi and bacteria causing plant diseases (Dhedi *et al.*, 1990; Alabouvette, 1990; Phae *et al.*, 1992; Dormann, 1993 and Mansour, 1997).

Table (1): Effect of different biocontrol agents on damping-off and root-rot diseases of tomato plants caused by *Fusarium oxysporum* f. sp. *lycopersici* under greenhouse conditions during 1997 and 1998 seasons.

Treatments	Effect of different biocontrol agents on disease incidence during					
	1997 season			1998 season		
	% post emergence damping-off	% Root-rot	Disease index	% post emergence damping-off	% Root-rot	Disease index
<i>Trichoderma harzianum</i>	19.11 ab	31.33 b	21.77 b	16.33 a	33.00 b	19.93 a
<i>Gliocladium virens</i>	20.55 ab	37.44 c	22.33 b	22.15 b	35.66 b	24.63 b
<i>Pseudomonas fluorescens</i>	23.89 b	48.44 d	26.44 c	27.72 c	51.55 c	30.33 c
<i>Bacillus subtilis</i>	23.33 b	51.33 d	26.33 c	30.88 c	55.44 d	33.33 d
<i>Actinomycete sp.</i>	24.311 b	48.11 d	25.00 c	30.11 c	48.00 c	30.98 c
Benomyle	13.33 a	27.66 a	16.66 a	16.66 a	25.66 a	18.91 a
Control	60.00 c	78.88 e	63.33 d	57.51 d	80.00 e	68.66 e

Means followed by a common letter in the same column are not significantly different at the 5% level.

Level of protection achieved was compared with those obtained by the application of the recommended dose of the fungicide benomyle. However, *T. harzianum* and *G. virens* were more effective than the other tested antagonists and gave protection against the tested soil-borne pathogens more or less to those obtained by benomyl. Numerous fungi, have been documented as effective antagonists against several important soil-borne pathogens. It has been reported that *T. harzianum* and *G. virens* showed strong antagonistic activity to *F. oxysporum* f. sp. *lycopersici* and

Phytophthora cinnamomi by mycoparasitism and over grew the pathogens (Cipriano et al., 1989 and Chambers and Scott, 1995).

Table (2): Effect of different biocontrol agents on damping-off and root-rot diseases of tomato plants caused by *Rhizoctonia solani* under greenhouse conditions during 1997 and 1998 seasons.

Treatments	Effect of different biocontrol agents on disease incidence during					
	1997 season			1998 season		
	% post emergence damping-off	% Root-rot	Disease index	% post emergence damping-off	% Root-rot	Disease index
<i>Trichoderma harzianum</i>	20.33 b	33.66 a	22.33 ab	19.33 a	39.66 b	22.66 a
<i>Gliocladium virens</i>	21.66 b	39.13 b	29.00 c	24.47 b	40.33 b	26.55 b
<i>Pseudomonas fluorescens</i>	27.33 c	55.66 c	30.33 c	29.33 c	44.55 c	30.44 c
<i>Bacillus subtilis</i>	33.00 d	55.33 c	35.33 d	30.33 c	51.36 d	33.22 c
<i>Actinomycete sp.</i>	30.33 cd	58.91 c	39.00 d	33.33 d	55.44 d	37.66 d
Benomyle	15.33 a	29.00 a	18.33 a	18.93 a	30.66 a	20.33 a
Control	78.89 e	95.88 d	85.18 e	70.81 e	88.66 e	70.00 e

Means followed by a common letter in the same column are not significantly different at the 5% level.

Table (3): Effect of different biocontrol agents on damping-off and root-rot diseases of tomato plants caused by *Fusarium solani* under greenhouse conditions during 1997 and 1998 seasons.

Treatments	Effect of different biocontrol agents on disease incidence during					
	1997 season			1998 season		
	% post emergence damping-off	% Root-rot	Disease index	% post emergence damping-off	% Root-rot	Disease index
<i>Trichoderma harzianum</i>	15.44 ab	27.00 a	16.33 a	20.92 a	39.33 b	22.00 e
<i>Gliocladium virens</i>	15.98 ab	33.33 b	19.77 ab	23.66 b	40.33 c	26.66 b
<i>Pseudomonas fluorescens</i>	19.89 c	41.44 c	22.22 b	27.55 c	51.44 d	30.33 c
<i>Bacillus subtilis</i>	22.33 c	55.44 d	25.66 b	30.00 d	51.33 d	33.33 c
<i>Actinomycete sp.</i>	25.00 c	51.33 d	30.33 c	28.77 c	55.44 d	33.33 c
Benomyle	13.92 a	26.00 a	13.33 a	19.44 a	30.33 a	20.33 a
Control	60.24 d	88.88 e	70.77 d	62.58 e	80.65 e	65.33 d

Means followed by a common letter in the same column are not significantly different at the 5% level.

The obtained results might be applied for controlling soil-borne pathogens instead of the fungicide in the field to avoid risks of using chemicals. These results are in general agreed with those of Khalifa (1991); Gamliel and Katan (1993); Benhamou *et al.* (1996); Khalifa and Liddell (1996) and Haggag, 1998.

Table (4): Effect of different biocontrol agents on damping-off and root-rot diseases of pepper plants caused by *Rhizoctonia solani* under greenhouse conditions during 1997 and 1998 seasons.

Treatments	Effect of different biocontrol agents on disease incidence during					
	1997 season			1998 season		
	% post emergence damping-off	% Root-rot	Disease index	% post emergence damping-off	% Root-rot	Disease index
<i>Trichoderma harzianum</i>	20.66 b	33.66 b	24.33 b	15.33 a	30.33 a	19.55 a
<i>Gliocladium virens</i>	23.33 b	44.44 c	30.33 c	19.66 b	37.33 b	25.33 b
<i>Pseudomonas fluorescens</i>	27.66 c	48.55 d	33.66 c	24.33 c	41.44 c	28.44 b
<i>Bacillus subtilis</i>	30.33 c	55.66 e	33.33 c	26.55	51.55 d	33.33 c
<i>Actinomyceete sp.</i>	25.33 c	51.44 d	30.33 c	24.66 c	48.44 d	60.66 bc
Benomyle	15.66 a	27.13 a	19.88 a	13.33 a	29.33 a	18.11 a
Control	69.00 d	92.33 f	85.33 d	67.88 d	88.11 e	79.88 d

Means followed by a common letter in the same column are not significantly different at the 5% level.

Table (5): Effect of different biocontrol agents on damping-off and root-rot diseases of pepper plants caused by *Pythium aphanidermatum* under greenhouse conditions during 1997 and 1998 seasons.

Treatments	Effect of different biocontrol agents on disease incidence during					
	1997 season			1998 season		
	% post emergence damping-off	% Root-rot	Disease index	% post emergence damping-off	% Root-rot	Disease index
<i>Trichoderma harzianum</i>	17.33 a	30.33 a	20.66 a	19.66 b	37.55 b	22.11 a
<i>Gliocladium virens</i>	20.33 a	37.44 b	25.66 b	20.44 b	42.55 c	25.33 b
<i>Pseudomonas fluorescens</i>	26.66 b	45.44 c	30.74 c	26.88 c	55.66 d	33.33 c
<i>Bacillus subtilis</i>	32.33 c	51.55 d	35.11 d	30.66 d	55.88 d	33.33 c
<i>Actinomyceete sp.</i>	28.33 b	51.44 d	33.33 cd	26.66 c	51.44 d	30.33 c
Benomyle	15.33 a	29.66 a	19.00 a	16.66 a	29.11 a	19.66 a
Control	85.55 d	95.33 e	88.18e	83.19 e	96.14 e	85.13 d

Means followed by a common letter in the same column are not significantly different at the 5% level.

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الملخص العربي
المكافحة الحيوية لبعض مسببات المرضيه المحموله بالتربة
والتي تصيب نباتات الطماطم والفلفل

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تم استخدام الفطريات ترايكودرما هارزيانم ، جليوكلاديوم فيرنز ،
والبيكتريات سيدوموناس فلورسنس ، باسيلس ساتس و عزلة اكتينومييسس فى
المقاومة الحيوية لبعض مسببات المرضية القاطنة بالتربة والتي تسبب الذبول
واعفان الجذور لنباتات الطماطم والفلفل. واستخدمت هذه الكائنات المختبرة تحت
ظروف الصوبة ، وظهرت النتائج المتحصل عليها انخفاض معنوى فى نسبة
تساقط البادرات بعد ظهورها فوق سطح التربة لنباتات الطماطم المتسببه عن
الفطرين فيوزاريوم اوكسيسورم ورايزوكتونيا سولانى. كما أظهرت هذه الكائنات
نتائج مشابهة على نباتات الفلفل التى عدت بكل من فطريات الفيوزاريوم سولانى
ورايزوكتونيا سولانى وبيثسيوم افانيسيدرماتم. وقد اوضحت النتائج أن الفطرين
ترايكودرما هارزيانم وجليوكلاديوم فيرنز أعطيا أفضل النتائج فى المقاومة الحيوية
لامراض الذبول وأعفان الجذور لكل من الطماطم والفلفل.