

IN VITRO EFFECTS OF SOME BACTERIAL AND FUNGAL ANTAGONISTS ON CERTAIN SOIL BORNE FUNGI ISOLATED FROM DISEASED TOMATO AND PEPPER PLANTS.

By

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ABSTRACT

Soil samples collected from the rhizosphere of healthy tomato and pepper plants resulted in isolation of 45 bacterial isolates and 12 fungal isolates exhibiting marked antifungal activity against *Fusarium oxysporum* f. sp. *Lycopersici*, *Rhizoctonia solani*, *Fusarium solani*, and *Pythium aphanidermatum* the causal fungi of wilt and root rot to tomato and pepper plants. These biocontrol agents belong to *Bacillus* spp., *Pseudomonas* spp. as bacterial antagonists as well as Actinomycetes isolates, while fungal isolates belonged to *Trichoderma* spp., *Gliocladium* spp., *Myrothecium* spp., *Paecilomyces* spp. and *Geotrichum* spp. Among these antifungal antagonists *Trichoderma harzianum*, *Gliocladium virens*, *Pseudomonas fluorescens*, *Bacillus subtilis* and an *Actinomycete* sp. proved to have high potential effect against the tested pathogens. Culture filtrates of the most tested antagonists significantly reduced the growth of the tested pathogenic fungi when added to PDA medium. The higher the increase in the filtrate concentration, the more the effect on the tested pathogenic fungi.

The antibiotic gliotoxin was found to be produced by either *G. virens* or *T. harzianum*. Such antibiotic proved an inhibitory action against all the tested pathogenic fungi.

INTRODUCTION

Biological control of soil-borne pathogens, manipulated in the last 15 years was used to avoid the ecological restrictions of using fungicides.

It has long been recognized that the biological control provides the front-line defence for roots against attacking by pathogens (Baker, 1986 and Bochow, 1989).

The primary approaches to evaluate the biocontrol antagonists against soil-borne plant pathogenic fungi are to demonstrate some direct adverse effects on the pathogen mycelium or on the physiology and or ecology of the pathogen caused by an antagonist metabolites (Dennis and Webster, 1971a,b).

Several antagonistic bacterial, fungal and *Actinomycetes* genera to soil-borne pathogens were reported as biocontrol agents of many pathogens

induced root-rot and wilt diseases (Duuff *et al.*, 1995; Benhamou and Chet, 1996 and Haggag, 1998).

It has been reported that *Bacillus* spp. inhibited the tomato wilt pathogen caused by *F. oxysporum* f. sp. *Lycopersici* by producing antifungal antibiotics in culture (Kapoor and Kar, 1989). They added that culture broth as well as cell free filtrates of 4 potent *Bacillus* isolates had an inhibitory effect.

Several antibiotics were produced by *Pseudomonas* spp. which inhibited growth of many soil-borne fungi.

Antibiosis is potentially a principal component of mechanism of the biocontrol by *Trichoderma* spp. and *Gliocladium* spp., *G. virens* produced an array of metabolites which were identified as antifungal and antibacterial compounds, i.e., viridin, sesquiterfen, gliotoxin, gliovirin, gliocladic acid, heptelicidic acid (avocetin), viridiol and valinotricin (Smith *et al.*, 1990).

The present work dealt with the antagonistic effects of certain biocontrol agents on some soil-borne pathogens isolated from roots and stems of diseased tomato and pepper plants. Also, to study the biochemical aspects of cultural filtrates of some of these bioagents.

MATERIALS AND METHODS

I. Screening for biocontrol agents:

Isolation of antagonistic fungi and bacteria were originally isolated from rhizosphere of healthy root systems of tomato and pepper plants by collecting adhering soil from the root system. Ten grams of such soil were added to 90 ml sterilized distilled water in conical flask (250 ml). After thoroughly shaking for 10 min., dilution series up to 10^8 CFU/ml was prepared. volumes of 0.1 ml from serial dilutions of the obtained suspension were spread on the surface of Petri dishes containing media using sterilized dryglasky glass triangle (Suslow and Schroth, 1982). Plates were incubated at 28°C for 1-3 days. To isolate the bacterial antagonist(s), nutrient agar and king's B media were used (King *et al.*, 1954 and Waksman, 1957). For isolating fungal antagonist(s) the method recommended by Elad *et al.* (1980) was followed. Actinomycete(s) was isolated on starch nitrate agar medium (Waksman, 1957).

Actinomycetes were checked for colony growth while fungi were checked after four days. Different separated bacterial or fungal colonies were picked up, repurified and stored on slants containing the suitable media in a refrigerator for further study of antagonism.

The bacterial and Actinomycetes antagonists were tested by streaking 2 cm-long on one side of the medium within Petri dish (9 cm diameter). Pathogen disks (6 mm diameter) were taken from 7 day-old cultures were put on the opposite side of the Petri dish.

All plates were inoculated at 28°C. Three replicates were used for each test. The radius of inhibition zone between the antagonist(s) and the pathogenic fungus was measured for dual culture plates when the fungus had completely covered the control plates as described by Ibrahim *et al.* (1997).

Potato dextrose agar medium (PDA) was used for isolating fungal antagonists. Each of the isolated pathogenic fungi i.e. *Fusarium oxysporium* f. sp. *lycopersici*, *Rhizoctonia solani*, *Fusarium solani* and *Pythium aphanidermatum* (6 mm discs) from 7 days old culture previously isolated by Hammoud (2000) from diseased tomato and pepper plants. Opposite to the pathogenic fungus, a disc of 7 days-old culture of the antagonist to be tested was placed at a constant distance away from the opposite edge of the Petri dish. Inoculated plates were incubated at 28°C for 7 days. Degree of antagonistic effect was scored according the scale adopted by Bell *et al.* (1982).

The bacterial and fungal antagonists were identified at the Dept. of Agric. Botany, Faculty of Agriculture, Kafr El-Sheikh as well as at the Plant Pathology, Institute, Agriculture Research Center, Giza, Egypt.

Effect of culture filtrates of the different bioagents on growth of the tested pathogens:

Different liquid medias, i.e., PD. amended with 0.2% yeast extract was used for fungi, nutrient glucose media, King's broth media were used for bacteria and starch nitrate media was used for Actinomycetes. Each bioagent isolate was inoculated in 250 ml flask contained 50 ml of specific medium and incubated at 28°C for 15, 6 and 10 days for fungi, bacteria and Actinomycetes, respectively, with continuous shaking conditions. Colonized media were filtered through sterilized membrane (0.45 µm mesh) (Lifshitz *et al.*, 1986). The clear filtrates were used as follow:

Aliquots of 0.00, 0.10, 0.25 and 0.50 ml were mixed with 0.50 ml of potato dextrose yeast extract agar (Abd El-Moity *et al.*, 1982 and Lumsden *et al.*, 1992). Sterilized medium containing filtrate of each antagonists was poured into Petri-dishes and inoculated with discs (6 mm diameter) obtained from 7-day-old colony of each pathogenic fungal growth. Plates without any culture filtrate were used as control and incubated at 28°C. Linear growth of each of the tested pathogens was measured until the pathogens in the control plate reached the edges. The inhibition zone were measured according to Klemant *et al.* (1990).

Metabolites produced by certain bioagents:

Detection and extraction of the antifungal compound(s) of *G. virens* and *T. harzianum*:

The antibiotic produced by either *G. virens* or *T. harzianum* *in vitro* was measured by growing each fungus in 50 ml of liquid media (Park *et al.*,

1992) and incubated at 28°C for 15 days on a rotary shaker at 60 rpm for 20 minutes. The cultures of the bioagents were then centrifuged at 16,000 g for 10 min. The antifungal compound of each was extracted with an equal volume of 80% aqueous acetone which was removed in vacuo. Aqueous residue was extracted with an equal volume of chloroform, which was removed in vacuo, and the residue was dissolved in 2 ml of methanol. Samples were spotted (30 µl) on thin layer chromatography plates of silica gel (200 µm thick) and developed in chloroform/ethyl acetate solvent (7: 3). Developed plates were observed under 366 and 254 nm ultraviolet light. R_F values of the spots were compared with metabolites extracted from bioagents and purified gliotoxin was used as standard (from sigma) (Howell, 1991). The extract was assayed for its antifungal activity by mixing 40 µl of extract with an equal volume of sterile water and placing the mixture in wells cut into the peripheries of agar in Petri dishes. Potato dextrose agar (PDA) plugs from 7 days old cultures of the pathogens were placed in the center of the dishes. After 2-6 days, the dishes were examined for the presence of clear zones around the wells. Each dish contained three replicate extracts and the dishes were arranged in completely randomized design.

Statistical analysis:

Complete randomized design was applied to laboratory experiments. Data were subjected to analysis of variance according to Duncan (1995) using the computer program (IRRISTAT).

RESULTS AND DISCUSSION

I. The *in vitro* experiments:

The initial screening of more than 250 bacterial colonies originated from different soil rhizosphere samples resulted in the isolation of 45 different bacterial isolates exhibiting obvious antibiosis against one or more of the tested pathogenic fungi. Each of the selected isolates were tested for purity and designated with a code number.

Results of physiological and biochemical testes indicated that 33 of the antagonistic isolates were aerobic Gram (+) and spore forming which proved to be identical to *Bacillus subtilis*. Nine isolates were pigment producer Gram (-), aerobic and short rods, which were identical to *Pseudomonas fluorescens*. While 3 isolates proved to belong to Actinomycetes spp. (Tables 1, 2).

The effect of the selected antagonistic bacteria on the tested phytopathogenic fungi were determined using a standardized test.

Table (1): Morphological characteristics and biochemical activities of the antagonistic isolate (B₅) identified as *Bacillus subtilis*.

Tests	Results
Shape of cell	Rods
Sporulation, spore shape	+, oval
Motility	Motile
Anaerobic growth	-
Gram reaction	+
Citrate utilization	+
V.P. reaction	+
Lecithinase production (LV reaction)	-
Nitrate reduction	+
Indole formation	-
Growth in 7% NaCl	+
Urease activity	+
Gelatin hydrolysis	+
Casein hydrolysis	+
Catalase reaction	+
Starch hydrolysis	+
Fermentation reaction:	
Glucose	Acid
Sucrose	Acid
Galactose	Acid

+ Positive reaction - Negative reaction

Table (2): Morphological characteristics and biochemical activities of the antagonistic isolate (P₃₅) identified as *Pseudomonas fluorescens*.

Tests	Results
Shape of cell	Short rods
Sporulation	Non-spore former
Motility	Motile
Gram reaction	-
An aerobic growth	-
Gelatin hydrolysis	-
Oxidase test	+
Growth of KBA medium	Production of fluorescent pigment
Casein hydrolysis	-
Starch hydrolysis	-
Fermentation reaction:	
Glucose	Acid
Sucrose	Acid
Galactose	Acid

+ Positive reaction - Negative reaction

Data presented in Table (3) show that some of the antagonistic isolates of *B. subtilis* had limited inhibitory spectrum namely isolate no. B₁, B₂₇ and B₇₈ which inhibited growth of *R. solani*, *F. oxysporum* f. sp.

Lyopersici and *F. solani* respectively. Similarly *P. fluorescens* isolate no. 19 inhibited growth of *R. solani* only. On the other hand, some of the antagonists had a wide spectrum of inhibitory action capable to inhibit all the tested pathogenic fungi namely isolates of *B. subtilis* no. 5, 8, 13, 18, 24, 33, 35, 44 and 51 as well as isolates of *P. fluorescens* no. 5 and 35. However, isolate no. 5 of *B. subtilis* was the best antagonist among all isolates of such a bacteria. Also, the obtained results show that two isolates of *Actinomycetes spp.* (no. 2 and 3) had limited inhibitory spectrum, while, isolate no. 1 was the best antagonist among all isolates of such micro-organism.

The obtained results agreed with the finding of many investigators who noticed the inhibitory effect of different *Bacillus spp.* particularly *B. subtilis* on many bacterial and fungal pathogens (Loeffler et al., 1986, Ferreira et al., 1991). Also, it was reported that *Pseudomonas spp.* inhibited different pathogens by producing antibiotics and/or siderophore which were suppressive to a large number of phytopathogenic fungi (Farvel, 1988 and Kraus and Loper, 1992).

Presence and role of *Actinomycetes spp.* in the rhizosphere of tomato and pepper plants as biocontrol agents are in accordance with the finding of other investigators (Saracchi et al., 1992 and Dormann, 1993).

The present study cleared that more than 200 fungal isolates were tested for their antagonistic effect on the tested phytopathogenic fungi. Twelve isolates out of these isolated fungi exhibited antagonistic effect against one or more of the tested pathogens. Six of these fungal isolates were identified as *Trichoderma spp.* One of them was identical to *T. harzianum*. Three isolates proved to belong *Gliocladium spp.*, whereas, one of them was identified as *G. virens*. The rest of these antagonistic fungi were *Paecilomyces sp.*, *Myrothecium sp.* and *Geotrichum sp.* (Table 4, 5), However, *T. harzianum* isolate proved to have the highest effect against all the tested pathogenic fungi while, *Trichoderma spp.* isolates no. T₂, T₄, T₅, T₆, showed moderate effect, while, T₃ had the least effect in this respect (Table 4).

Results also, show the inhibitory effect of *Myrothecium sp.*, *G. virens*, *Gliocladium spp.*, *Paecilomyces sp.* and *Geotrichum sp.* against the tested soil-borne fungal pathogens of tomato and pepper plants (Table 5). However, *G. virens* was the best antagonist. This finding is in accordance with (Bell et al., 1982; Papavizas, 1985 and Chamber and Scott, 1995).

Filtrates of antagonists were examined for their inhibitory action on all tested phytopathogenic fungi. It is clear from the data that culture filtrate of *T. harzianum* had the highest effect which inhibited mycelial growth of the different tested phytopathogenic fungi (Table 6).

Table (3): Relative power of antibiosis (RPA) of bacterial antagonists against the tested soil-borne fungal pathogens of tomato and pepper plants.

No.	Code No. of antagonistic isolates	Values of RPA of tested bacterial antagonists against pathogens infect:				
		Tomato plants		Pepper plants		
		<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	<i>Rhizoctonia solani</i>	<i>Fusarium solani</i>	<i>Rhizoctonia solani</i>	<i>Pythium aphanidermat um</i>
1	B ₈	2.21 bcd	1.91 e-h	2.18 cde	2.10 def	1.23 gh
2	B ₁₃	2.54 ab	2.17 de	2.36 bc	3.25 a	1.78 cd
3	B ₃₅	2.19 bcd	1.87 e-i	1.98 d-g	2.35 cd	1.49 e
4	B ₁₈	1.83 d-i	1.72 g-j	1.79 f-i	1.23 pq	1.11 h
5	B ₅	2.99 a	2.52 bc	2.54 ab	2.33 cd	2.71 a
6	B ₃₃	2.21 bcd	2.15 def	2.26 bcd	2.07 d-g	2.31 b
7	B ₄₄	1.75 d-i	1.86 e-i	1.95 efg	1.64 hn	1.91 c
8	B ₇₁	1.87 d-g	1.81 f-j	1.22 kl	0.00 r	0.00 i
9	B ₅₅	1.97 c-f	1.53 i-n	1.38 jkL	1.25 pq	0.00 i
10	B ₇₈	0.00 j	0.00 p	1.21 L	0.00 r	0.00 i
11	B ₆₉	1.46 f-i	1.28 mno	1.59 hij	1.68 h-m	1.73 d
12	B ₂₃	2.91 a	0.00 p	1.78 ghi	0.00 r	0.00 i
13	B ₄₇	1.77 d-i	0.00 p	2.80 a	0.00 r	0.00 i
14	B ₂₄	1.61 f-i	1.47 j-o	1.77 ghi	1.86 f-j	1.45 ef
15	B ₁₅	1.35 hi	1.31 L-o	1.92 efg	1.27 opq	0.00 i
16	B ₁₆	1.77 d-i	1.48 j-o	1.28 kL	0.00 r	0.00 i
17	B ₁₀₅	2.13 b-e	1.85 e-i	2.31 bc	0.00	1.37 efg
18	B ₁₀₆	1.59 f-i	1.32 L-o	1.94 efg	1.75 g-m	0.00 i
19	B ₂	2.17 bcd	1.98 efg	2.10 c-f	1.80 f-k	0.00 i
20	B ₁	0.00 j	1.68 g-k	0.00 m	1.56 j-P	0.00 i
21	B ₉₉	1.39 ghi	0.00 p	0.00 m	0.00 r	0.00 i
22	B ₂₇	0.00 j	1.73 g-j	0.00 m	1.65 h-n	0.00 i
23	B ₁₇	2.96 a	3.21 a	2.72 a	2.87 b	0.00 i
24	B ₈₈	1.75 di	1.36 k-o	0.00 m	1.352 n-q	0.00 i
25	B ₆₈	1.97 c-f	0.00 p	1.84 fgh	1.11 q	1.34 efg
26	B ₅₁	1.85 d-h	1.78 g-j	1.92 efg	1.15 q	1.22 gh
27	B ₃₀	1.32 i	1.16 o	1.61 hij	1.96 e-h	0.00 i
28	B ₉₁	1.63 e-i	1.66 g-i	0.00 m	2.45 c	0.00 i
29	B ₁₂₅	2.44 bc	2.19 de	1.92 efg	0.00 r	0.00 i
30	B ₁₃₇	0.00 j	1.28 mno	0.00 m	1.59 i-o	0.00 i
31	B ₈₉	0.00 j	2.3 9 cd	0.00 m	1.78 f-L	0.00 i
32	B ₆₀	0.00 j	1.69 g-k	0.00 m	2.21 cde	0.00 i
33	B ₁₉₀	0.00 j	1.25 no	0.00 m	0.00 r	0.00 i
34	P ₅	1.32 i	1.61 h-m	1.61 hij	1.46 L-q	1.28 fgh
35	P ₃₅	2.54 ab	2.76 b	2.82 a	2.92 b	2.63 a
36	P ₂₅₀	1.87 d-g	1.92 e-h	1.98 d-g	1.68 h-m	0.00 i
37	P ₂₁₀	0.00 j	0.00 p	1.58 hij	1.72 h-m	0.00 i
38	P ₈₇	1.48 f-i	1.24 no	1.36 jkl	1.33 n-q	0.00 i
39	P ₁₉	0.00 j	0.00 p	1.51 ijk	1.91 e-i	1.28 fgh
40	P ₁₇	1.79 di	1.88 e-i	0.00 m	1.44 mq	0.00 i
41	P ₇₂	0.00 j	0.00 p	1.12 L	0.00 r	0.00 i
42	P ₁₀₈	0.00 j	1.87 e-i	0.00 m	0.00 r	0.00 i
43	Act. 1	2.80 a	3.01 a	2.53 ab	2.85 b	2.68 a
44	Act. 2	0.00 j	1.86 e-i	0.00 m	1.48 k-q	0.00 i
45	Act. 3	0.00 j	1.21 no	0.00 m	1.20 q	0.00 i

In the same column mean followed by the same letter are not significantly different according to DMRT at 0.05 level.

Table (4): Effect of *Trichoderma* spp. against the tested phytopathogenic fungi of tomato and pepper plants.

Isolates of <i>Trichoderma</i> spp.	Values of antibiosis of <i>Trichoderma</i> spp. against pathogens of:*				
	Tomato plants		Pepper plants		
	<i>Fusarium oxysporum</i> f. <i>sp. lycopersici</i>	<i>Rhizoctonia solani</i>	<i>Fusarium solani</i>	<i>Rhizoctonia solani</i>	<i>Pythium aphanidermatum</i>
<i>Trichoderma harzianum</i>	1	2	1	2	1
<i>Trichoderma</i> sp. (2)	2	3	3	2	2
<i>Trichoderma</i> sp. (3)	2	4	4	4	3
<i>Trichoderma</i> sp. (4)	3	2	4	3	2
<i>Trichoderma</i> sp. (5)	4	3	3	2	1
<i>Trichoderma</i> sp. (6)	3	3	3	3	1

*1 = Antagonist completely over grew the pathogen.

2 = Antagonist over grew at least two thirds of medium surface.

3 = Antagonist and pathogen each colonized approximately one half of medium surface.

4 = The pathogen colonized at least two thirds of medium surface.

5 = The pathogen completely over grew the antagonist and occupied the entire medium surface.

Table (5): Effect of different fungal antagonists against the tested soil borne fungal pathogens of tomato and pepper plants.

Fungal antagonistic isolates	Relative power antibiosis (RPA)				
	Pathogens of tomato		Pathogens of pepper		
	<i>Fusarium oxysporum</i> f. <i>sp. lycopersici</i>	<i>Rhizoctonia solani</i>	<i>Fusarium solani</i>	<i>Rhizoctonia solani</i>	<i>Pythium aphanidermatum</i>
1. <i>Myrothecium</i> sp.	0.00 d	0.87 c	0.80 c	1.43 c	1.83 ab
2. <i>Gliocladium virens</i> (1)	2.54 a	2.20 a	3.09 a	2.36 a	2.13 a
3. <i>Gliocladium</i> sp.	1.75 bc	1.78 b	2.47 a	1.91 b	1.89 ab
4. <i>Gliocladium</i> sp.	1.85 bc	1.78 b	1.78 b	1.71 c	2.10 a
5. <i>Paecilomyces</i> sp.	1.52 c	1.20 c	1.10 c	0.75 d	1.38 b
6. <i>Geotrichum</i>	0.00 d	1.25 c	0.78 c	0.89 d	0.88 c

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

$$RPA = \frac{z}{c}$$

z = Diameter of inhibition zone.

c = Diameter of fungal antagonistic isolate.

It was found that the more the increase in filtrate concentration, the higher the effect on the pathogenic fungi.

The inhibition of pathogenic fungi when grown on media included culture filtrate of each of the tested antagonists was probably due to the presence of fungitoxic compounds or antibiotic compound(s) which could

affect fungal growth. The production of toxic metabolites by bioagents is the principal mechanism of biological control of root-rotting pathogens. These results are in a harmony with Roberts and Lumsden (1990) and Haggag, Wafaa, (1998).

Table (6): Effect of culture filtrates of different antagonistic microorganisms on percent of fungal pathogens growth of tomato and pepper plants.

Antagonists	Filtrate conc.	% fungal growth reduction of pathogens of				
		Tomato plants		Pepper plants		
		<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	<i>Rhizoctonia solani</i>	<i>Fusarium solani</i>	<i>Rhizoctonia solani</i>	<i>Pythium aphanidermatum</i>
<i>Trichoderma harzianum</i>	0	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d
	1	28.54 c	18.09 c	35.21 c	14.28 c	27.33 c
	2	51.90 b	35.23 b	49.52 b	33.80 b	39.52 b
	3	58.51 a	89.52 a	58.09 a	82.38 a	71.90 a
<i>Gliocladium virens</i>	0	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d
	1	20.47 c	18.57 c	27.14 c	17.61 c	17.61 c
	2	49.52 b	42.85 b	38.57 b	39.52 b	36.66 b
	3	53.33 a	74.76 a	56.19 a	74.95 a	75.71 a
<i>Pseudomonas fluorescens</i>	0	0.00 d	02.00 d	0.00 d	0.00 d	0.00 d
	1	22.09 c	28.52 c	20.76 c	17.61 c	27.14 c
	2	45.33 b	48.23 b	39.14 b	30.52 b	32.59 b
	3	65.52 a	82.38 a	69.19 a	76.95 a	80.19 a
<i>Bacillus subtilis</i>	0	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d
	1	22.38 c	25.23 c	25.71 c	27.14 c	26.18 c
	2	35.71 bc	46.66 b	42.85 b	39.66 b	30.23 bc
	3	55.23 a	68.15 a	48.09 b	65.71 a	69.52 a
<i>Actinomycece sp.</i>	0	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d
	1	40.95 bc	51.43 c	41.90 c	20.95 c	48.38 c
	2	58.57 b	68.95 b	50.95 b	41.19 b	60.47 b
	3	67.14 a	59.52 a	61.42 a	76.65 a	68.43 a

Means followed by a common letter in the same column are not significantly different at the 0.05 level by DMRT.

II. Detection and extraction of the antifungal compound(s) extracted from *gliocladium virens* and *Trichoderma harzianum*:

Identification of antifungal compound(s) produced by either *G. virens* or *T. harzianum* using thin layer chromatography technique indicated the existence of the antibiotic Gliotoxin by both fungi (Fig. 1). The solvent system, chloroform ethyl acetate (7: 3) was the most effective in separating the compound. The metabolite was particularly prominently visible in short wave UV/250 nm) at RF value at (92.1).

III. Effect of Gliotoxin on mycelial growth of certain phytopathogenic fungi:

Gliotoxin was assayed for its effect on the tested pathogenic fungi isolated from diseased tomato and pepper plants. Data in Table (7) indicated that, Gliotoxin showed an inhibitory action on the tested pathogenic fungi.

However, *R. solani* was the most affected pathogen by the antibiotic while *Fusarium solani* was the least affected one. It was also clear from the data that concentration of the antibiotic extracted from *G. virens* was higher than those extracted from *T. harzianum*.

In the present study gliotoxin is likely to be responsible for biocontrol activity *in vitro* effect against soil-borne pathogenic fungi. This result is in agreement with Howell (1982 and 1991); Lumsden *et al.* (1992) and Howell and Stipanovic (1995) who cited that the gliotoxin serve to inhibit soil microbial competitors, and affect germination and growth of soil-borne pathogens to bring about biological control. Gliotoxin is a major component of the biocontrol systems of *G. virens*.



Fig. (1) Detection and extraction of *Gliotoxin* from either *G. virens* or *T. harzianum* by thin layer chromatography

A: Gliotoxin (standard)

B: *G. virens*

C: *T. harzianum*

Table (7): Effect of Gliotoxin produced by either *Trichoderma harzianum* or *Gliocladium virens* on the tested pathogenic fungi of tomato and pepper plants.

Antagonists	Relative power antibiosis (RPA) against fungal isolates of				
	Tomato plants		Pepper plants		
	<i>Fusarium oxysporum</i> f. sp. <i>lycopersici</i>	<i>Rhizoctonia solani</i>	<i>Fusarium solani</i>	<i>Rhizoctonia solani</i>	<i>Pythium aphanidermatum</i>
<i>T. harzianum</i>	2.24 b	2.49 ab	1.49 c	2.09 b	2.93 a
<i>G. virens</i>	2.77 b	3.36 a	1.68 c	3.01 a	2.24 b
Control (without Gliotoxin)	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d

In the same row means followed by the same letter are not significantly different according to DMRT.

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الملخص العربي

دراسات معملية عن تأثير بعض البكتريات والفطريات المضادة على بعض المسببات الفطرية المحمولة بالتربة والمعزولة من نباتات الطماطم والفلفل المريضة

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تم عزل ٤٥ عزله بكتيرييه و ١٢ عزله فطريه من عينات التربة التى تم جمعها من المنطقة المحيطة بجذور نباتات الطماطم والفلفل السليمه والتى أظهرت نشاطا تضاديا واضحا ضد بعض الفطريات الممرضة لنباتات الطماطم والفلفل مثل فيوزاريوم اوكسيبورم ورايزوكتونيا سولاني وفيوزاريوم سولاني وبيثيوم افانيدرماتم والتى تحدث مرض الذبول وعفن الجذور فى نباتات الطماطم والفلفل.

كانت البكتريات المعزولة المضاده لنمو الفطريات الممرضة تندرج تحت الاجناس باسيلس *Bacillus spp.* وسيدوموناس *Pseudomonas spp.* والاكثينوميستات *Actinomycetes spp.* ، أما الفطريات المضاده لنمو الفطريات الممرضة تندرج تحت اجناس ترايكودرما *Trichoderma spp.* وجليوكلاديوم *Gliocladium spp.* ، مسيروثييوم *Myrothecium spp.* وباسيلومايسيس *Paecilomyces spp.* وجيوتريكام *Geotrichum spp.*

اوضحت النتائج ان ترايكودرما هارزيانم – جليوكلاديوم فيرز – سيدوموناس فلورسنس وباسيلس ساتلس واكتينوميستيس كانت اكفأ الكائنات المضاده للمسببات المرضيه. وباضافة راسح هذه الكائنات المضاده الى البيئة الغذائية للمسببات المرضيه ادى الى تثبيط نمو المسببات المرضيه معنويا ، وازداد هذا التثبيط بزيادة تركيز الراشح.

تم استخلاص المضاد الحيوى جليوتوكسين *Gliotoxin* وهو احد المنتجات الايضيه للفطرين جليوكلاديوم فيرنز وترايكودرما هارزيانم والذى كان له دورا فى تثبيط جميع الفطريات الممرضة المختبرة.