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BIOLOGICAL CONTROL OF ROOT-ROT AND WILT DISEASES OF PEPPER PLANTS

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ABSTRACT

Rhizosphere colonizing bacteria and fungi were counted and isolated from samples of six crops grown in reclaimed fields at Mariout districts. A total of 69 bacterial and 22 fungal isolates in addition to four known bioagents were tested, *in vitro*, for their antagonistic effect against the agents of wilt and root rot of pepper namely *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani*. Twenty three isolates showed moderate to strong inhibition or hyperparasitism on mycelium of at least one of these pathogens The biocontrol ability of these antagonists isolates was evaluated using an *in vivo* assay. Accordingly, six isolates were selected and used for soil treatment to control root rot and wilt of pepper in pot experiments. The most effective isolates were *Pseudomonas fluorescens* (BA1), followed by *T. harzianum* (Th1) and reduced root rot or wilt incidence by not less than 75%. Significant increase in fresh and dry weight of pepper plants treated by *P. fluorescens* or *T. harzianum* were obtained in pathogens free soils. Treatment of pepper transplants by both BA-1 and Th-5 isolates, before transplanting in the field, significantly reduced incidence of root rot and wilt of pepper, however, isolate Th5 had the dual effect of disease suppression as well as growth and yield increase.

Key words : Pepper, Root rot, Wilt, Biological control, *Trichoderma harzianum*, *Pseudomonas fluorescens*.

INTRODUCTION

Pepper (*Capsicum annum* L.) is one of the most important vegetable crops in Egypt, based on consumption, nutritional value and cash value to farmers. Pepper plants are liable to attack by several soil borne pathogens, causing severe losses in yield and quality (Moens and Ben-Aicha, 1990; Hwang and Kim, 1995; Abada, 1994; Abd El-Kader, 1999 and Abd El-Naby, 2001). Producers commonly suffer deom losses due to death of seedlings or mature plants. Root rot and wilt diseases of pepper are primary caused by the ubiquitous pathogens *Rhizoctonia solani, Pythium* spp. and *Fusarium oxysporum* (Harfoush, 1970; Abada, 1994 and Abd El-Kader, 1999). These pathogens are capable of surviving in the soil in the absence of their host plants and when weather conditions are not favorable for disease initiation and development (Singleton *et al.*, 1992).

The main measure applied by growers to reduce losses due to these pathogens, in greenhouse, especially at the early stages of plant developmental are pre plant soil fumigation with methyl bromide and application of fungicides after planting (Jarvis, 1992). However, the use of methyl bromide has been banded in several countries for environmental

concerns. Lack of disease resistant varieties, high cost and inadequate protection by fungicides are the major obstacle in managing such pathogens (Attia and Abada, 1994) and have prompted a search for alternatives for use in the control of soil borne pathogens. One of such alternative is biological control using soil microorganisms that reduce the amount of inoculum or disease producing activity of pathogens (Cook, 1993). Successful biological control of several soil borne pathogens using various microbial antagonists including strains of *Trichoderma* species, fluorescent Pseudomonads and *Bacillus subtilis* were widely used worldwide (Nemec *et al.* 1996; Abd El-Ghafar *et al*, 1996 and Sid-Ahmed *et al.*, 1999).

The objectives of the present study was to search for antagonistic bacteria and fungi, in rhizospheric soil that suppress soil borne of pepper; and to test promising strains for controlling root rot and wilt diseases under greenhouse and field conditions.

MATERIAL AND METHODS

Source of seeds :

Seeds of pepper (*Capsicum annum* L.) cv. California Wonder were obtained from commercial source in Egypt and used throughout this study. *Seeds were washed overnight in runing* tap water, just before sowing, and were surface sterilized in 2% sodium hypochlorite solution for 2 min.

Pathogens and inoculum

Pepper seedlings and plants, showing root rot and wilt symptoms, were collected from fields in different reclaimed locations in Egypt during *early summer and* summer seasons in 1997. *Fusarium oxysporum* (Schlecht.), *Pythium aphanidermatum* (Edson) Fitzp. and *Rhizoctonia solani* (Kühn) were frequently isolated and identified according to Booth (1971), *Plaats-Niterink* (1981) and Sneh *et al.* (1992) respectively. Pathogenicity tests of these isolates were performed and their pathogenic potentialities were proven. Purified isolates were maintained on potato dextrose agar (PDA) *medium of* 4°C till use.

Inoculum of each fungal isolate was prepared using ground corn or barley-grain medium in polyethylene bags, each containing 200 g medium as *described* by *Singleton et al.* (1992). Meanwhile, inoculum was prepared as spore suspension (10^4 spore/ml) for *F. oxysporum*, mycelial fragment suspension (10^4 cfu/ml) for *R. solani*, and suspension of hyphae, sporangia *and* oospores (10^4 cfu/ml) for *P. aphanidermatum*.

Source of antagonists

Rhizosphere-colonizing bacteria and fungi were isolated from samples of six crops, i.e. alfa-alfa, barley, garlic, maize, mustard and pepper, brought from fields at Mariout district in 1997. Dilutions from each soil sample were prepared plated in triplicate on four different media: triptic soy agar (TSA) for isolation of heterotrophic bacteria according to Gould *et al.* (1985), King's media B (KB) for isolation of fluorescent pseudomonads (King *et al*,

1954), and potato dextrose agar (PDA) supplemented with 100 µg/ml streptomycin sulfate for isolation of fungi. Plats were incubated at 28°C for 2-4 days when individual colonies were picked up, purified and stored at 4°C on the appropriate medium. In addition, four fungal and bacterial isolates previously proved biocontrol activity against soil-borne pathogens were also used. These isolates were *Trichoderma harzianum* (Th-5) provided by Prof. Dr. Ahlam M. Gowily, *Pseudomonas fluorescens* (Pf1) kindly supplied by Prof. Dr. Bosina F. Abdel Khaney Prof. of Microbiology, Soil Microbiology Dept. (DRC) and *Burkuldaria cepacia* (K1) and *Bacillus subtilis* (Bs1) kindly supplied by Dr. K. Zaki (Desert Research Center).

Assay of antagonism, *in vitro*

Dual-culture inhibition plates were performed on PDA medium, in 9 cm Petri dishes. Disks, 5-mm, diameter, of fungal pathogens were removed from the margin of 5 days-old cultures on PDA and placed 6 cm apart from the tested fungal isolate The plates were incubated at 22°C for 6-10 days, then examined macroscopically for evidence of antibiosis and/or microparasitism.

The bacterial isolates were tested as follows: each bacterial isolate was streaked in the center of culture plate containing PDA medium, then incubated for 48 h. at 25°C. Plates were then inoculated with each studied pathogen by placing two 5 mm diameter disks, from three days old culture of the fungus, 3 cm apart from both sides of bacterial growth. Plates were then incubated at 25°C, for 96 h. and fungal colony diameter in the presence or absence of bacteria were measured. The inhibition zone between bacteria and the pathogen colony was measured as described by Maurhofer *et al.* (1995).

Identification of antagonists

Identification of bacterial isolate BA1 as *Pseudomonas flourescens* was carried out according to standard biochemical and physiological tests described by Palleroni (1984) by the kind help of Dr. N.Y. Abd El-Ghaffar, Associate Prof., Plant Path. Dept., Fac. Agric., Ain Shams Univ.

Production of antagonists inoculum

Inocula of antagonistic fungal were prepared from cultures grown on PDA plates as conidial suspension (2 X 10^6 spores/ml) (Sivan *et al.*, 1984). Meanwhile, bacterial inocula were prepared as described by Mosa *et al.* (1997) to give bacterial suspension (10^8 cfu/ml).

Seed and soil treatments

Surface disinfested pepper seeds, were mixed throughly with two ml bacterial suspension (1X10^8 cuf/ml) or fungal spore suspension (2X10^6 conidia/ml) in 0.01% methyl cellulose in a small Petri dish. The seeds were air dried for 30 min in a Laminar-flow cabinet and were planted directly. Seeds treated with methyl cellulose were used as control.

Soil treatment by antagonists were carried out by adding ten ml of conidial suspension of each fungal isolate (2X10^6 spore/ml) or bacterial suspension (1X10^8 cfu/ml) to each kg soil 24 h before transplanting in the

pots or were added to each pepper transplant, 24 h before transplanting in the field.

Assay of antagonism *in vivo*

The most effective bacterial and fungal isolates in laboratory tests were tested, *in vivo*, against the pathogens *F. oxysporum*, *R. solani* and *Pythium aphandiermatum* using the soil-dishes technique described by Mosa *et al.* (1997). The pathogens were grown for five days on a thin layer of potato dextrose agar media, in 9-cm diameter petri dishes. Then, the fungal colony were covered by autoclaved mixture of peatmoss and vermiculite (1:1 V/V). Treated pepper seeds were sown over soil in each petri dish using sterile tweezers to prevent cross contamination through handling. Set of dishes contained non infested soil served as control. Treatment with the fungicide Rizolex-T (2 g/kg seeds) was carried out for comparison. Thereafter, seeds were covered by the soil mixture, watered daily by sterilized distilled water. Percentages of survived seedlings were recorded after twenty five days from sowing date. Seedling dry weights were also determined.

Efficiency of antagonists under greenhouse conditions

An experiment were carried out during 1999 summer season for studying the effect of the most promising antagonistic isolates, for controlling root-rot and wilt disease pathogens of pepper.

Plastic pots (25 cm diameter) containing sterilized sandy-loam soil were prepared. Soil was infested with inoculum of *P. aphanidermatum* grown on corn ml-sand medium at the rate of 5 g/kg soil or with 10 ml conidial suspension ($1X10^4$ spore/ml) (Kg/soil) of *F. oxysporum* or 5 g/kg soil of barley grain inoculum of *R. solani*. Inoculum of each pathogen was mixed thoroughly with soil. Infested pots were irrigated and left for 5 days before transplanting. The experiment included the following treatments: (a) non-infested soil (control), (b) Soil-treated with aqueous suspension of antagonists or water as control (c) Soil treated with the fungicide Rizolex-T aqueous suspension (500 µg active ingredient/ml water). Five seedlings were transplanted in each pot, three replicate pots were specified for each treatment. Pots were kept under greenhouse conditions till the end of the experiment. Percentage of infected plants with wilt and/or root-rot were recorded up to 8 weeks after transplanting.

For wilt disease, number of symptomatic leaves and dead plants were recorded after about 4 to 8 weeks from transplanting. Wilt development on each plant was rated using the scale described by **Gao *et al.* (1995)** as follows : 5= plant dead; 4 = 76 to 100% of leaves with symptoms, 3 = 51 to 75% of leaves with symptoms; 2 = 26 – 50% of leaves with symptoms; 1 = <25% of leaves; with symptoms; and 0 = no symptoms. The disease rating was calculated by the following formula:

$$\text{Disease index} = \frac{\Sigma \text{ (rating no. x no. of plants in the rating)} \times 100}{\text{Total no. of plants x highest rating}}$$

Internal symptoms were determined based on length of vascular discoloration (cm) as described by Szczech (1999).

Efficiency of antagonists in the field :

Field experiments were carried out at Mariout Experimental Research Station,Desert Research Center, during summer seasons, 2000, to study the effect of two promising isolates *T. harzianum* (Th5) and *P. fluorescens* (BA-1) for controlling root rot and wilt of pepper. Soil was calorious with pH 7.8. The experiment was applied in a split plot design (with the pathogen in the main plot and bioagent treatments in sub-plot). The experimental unit area was 5 m^2. Each unit included four rows, each row was 2.5 m in length and 50 cm width. Pepper transplants were sown at 10 seedlings within each row. During the growing season, convential cultural practices were followed except the subject under studying was used.

Pepper plants, 6 weeks old, grown in seedling multi-pot trays containing peatmoss-vermiculite mixture (1:1 W/W) were inoculated with 10 ml of (10^4 cfu or spore) of each tested pathogen for each transplant, before transplanting (Pamirez-Villapudus and Munnecke 1987). Meanwhile, each transplant was treated by drenching with 10 ml conidal suspension ($1x10^6$) of *T. harzianum* or in 10 ml bacterial suspensions (1×10^8 cfu/ml) of *P. flourescens* 24 h. before transplanting. A fungicide treatment was also included using "Rizolex-T (50 WP)", where 800 ml suspension (500 µg active ingredient/ml) was sprayed directly into each seedling tray. Control plants were treated with water only. Incidences of root rot and wilt diseases were recorded after 8 weeks from transplanting. Foliar wilt rating and length of vascular discoloration were determined as described previously. Meanwhile, fresh and dry weight of plants and fruit yield were also determined.

Determination of pathogen population in treated soil :

Soil-dilution plating techniques were used to monitor populations of pathogens as affected by biocontrol treatments. The appropriate soil dilutions were plated into three plates of different selective media namely Komad's medium (Komada, 1975) was used to isolate for *Fusarium oxysporum*; Gallic acid medium (Flowers and Hendrix 1969) *Pythium* sp., KO and Hora agar medium (Ko and Hora 1971) was for *Rhizoctonia solani*; plates were incubated at 28°C for 2-4 days, then developed individual colonies were examined and counted.

Statistical analysis :

Data were subjected to analysis of variance (ANOVA) using the Statistical Analysis System (SAS Institute, Inc., 1996). Means were separated by Duncan's multiple range test at P= 0.05 level.

RESULTS

Isolation of rhizosphere-colonizing microorganisms :

Rhizosphere-colonizing bacteria and fungi from six different plant species grown in reclaimed field at Mariout district in Egypt were counted. Data in Table (1) illustrate that, the highest bacterial counts were obtained from alfalfa rhizosphere followed by barley and maize. Meanwhile, total count

of fungi was also varied .The highest count was recorded with barley, mustard and pepper (Table 1). However, there was variation among the three tested media for isolation of bacteria from soil. For example, the highest count of bacteria were obtained from alfalfa on King B medium and on NA medium for barley. A total of 69 bacterial and 22 fungal isolates were isolated from rhizosphere of these plants and established in pure cultures. Most isolates were obtained from barley and alfalfa.

Table (1): **Total count of rhizobacteria and fungi from different plants grown in fields at Mariout district, on different agar media [a]:**

Source	Bacteria (cfux10^8)			Fungal (cfu x10^4)
	TSA	NA	King B	PDA
Alfalfa	9	3	11	3
Barley	4	11	5	5
Garlic	5	NT[b]	0.1	4
Maize	5	7	3	NT
Mustard	6	NT	1	5
Pepper	2	5	NT	5

i) TSA = Ttryptic soy agar medium, NA = Nutrient agar medium, King B= King's B medium, PDA = Potato dextrose agar medium.
b) cfu = colony forming unit/g dry soil.
c) NT = not tested

Antagonistic effect of selected fungi and bacteria, *in vitro*

Data in Table (2) summarizes the results of the *in vitro* assays by which 91 bacterial and fungal isolates, in addition to four known isolates, were evaluated for their antagonistic effect against *F. oxysporum, P. aphanidermatum* and *R. solani* on PDA medium. Only, 23 isolates caused moderate to strong inhibition to the three pathogenic fungi on plates. The results indicated that, inhibition zone was mainly observed between bacteria and tested pathogens, and measured from 2.3 to 27.3 mm (Table 2)., The most antagonistic bacterial isolates were BA-1 and Mu-3 which reduced mycelial growth of the three tested fungal pathogens isolates. Some fungal isolates, *Trichoderma harzianum* (Th-5), (P-10) and (P-12) showed hyperparasitism on hyphae of *P. aphanidermatum* and *R. solani*. While isolates Al-25 and GA-7 showed a moderate inhibition zone against the three pathogens.

Screening of antagonists, *in vivo*

Data in Table (3) indicate that the degree of reduction of damping off varied according to bacterial isolate and the pathogen. Three bacterial isolates i.e. BA1, *B. cepacia* (K1), and Mu3 and three fungal isolates i.e. *Trichoderma harzianum* (Th5), GA7 and AL-25 were effective in reducing damping-off of seedlings caused by *F. oxysporum* and *P. aphanidermatum*. However, seed treatment with the fungicides Rizolex-T significantly reduce damping-off caused by the three pathogens. Negative or positive effects were recorded zero seedlings as a result of treatment with bacterial and fungal antagonists. However, isolates (Al-2 and BA-7) caused deleterious effect and reduced seedling survival. Meanwhile, in non-infested soil isolate BA1 increased the seedling dry weight by 14% (Table 3).

Table (2): Antagonistic effects of selected rhizosphere colonizing bacterial and fungal isolates on growth of three pathogenic soil borne fungi on PDA medium.

Isolate and Source	Inhibition Zone		
	F.oxysporum	P.aphandermatum	*R. solani*
Known bioagent:			
T. hanzianum, (Th5)	11.2	+	+ [b]
P. fluorescence (Pf1)	18.5	12.3	11
B. cepacia (K1)	17.2	5.4	20.2
B. subtils (Bs1)	9.5	4.6	11.2
Field Isolates: [b]			
Fungi :			
AL-25	13.6	6.4	11.3
P-10	0	+ [c]	+
P-12	0	+	+
GA-7	16.2	5.1	13.3
Bacteria :			
AL-2	18.4	12.5	15.3
AL-6	6.0	6.5	2.5
AL-9	12.3	9.2	11.7
AL-19	8.5	7.3	9.2
BA-1	23.5	25.4	27.3
BA-7	12.5	16.2	14.5
BA-11	2.7	7.5	2.3
BA-15	19.5	11.7	13.2
MU-3	25	17.5	23.7
MU-6	12.7	9.2	7.2
ZE-1	19.3	17.6	15.7
ZE-5	3.7	2.2	2.9
ZE-9	12.7	6.8	9.2
ZE-13	9.7	5.6	8.3
ZE-14	21.2	19.5	18.2
Control	0	0	0

a)Inhibition of pathogens is expressed as the distance (mm) between pathogen mycelium and bacterial or fungal colony on potato dextrose agar (PDA), each value is the mean of three replicates.

b) Isolates code designted according to source plant as follows:AL=alfa alfa,P=pepper,GA=garlic,BA=barley,MU=mustard;ZE=maize.

c) Hyperparasitism was observed microscopically (+)

Suppression of root rot and wilt (Greenhouse Experiment):

Data in Table (4) indicate that, all tested bioagents except *Penicillium* sp. (AL-25) significantly reduced wilt and root-rot diseases. The most effective isolates were *Pseudomonas flourescens* (BA-1) and *T. harzianum* (Th-5) which reduced wilt incidence by 87.5% and root rot incidence by not less than 75%. Internal root browning and foliar wilt rating were significantly reduced by not less than *T. harzianum* compared to *Pseudomonas*

fluorescens treatment. The effect of both bioagents, *T. harzianum* (Th-5) and *Pseudomonas flourescens* (BA-1) , was superior to the fungicide Rizolex-T.

Results also indicate that, in sterilized non infested soil, the bioagent treatments varied in their effects on plant growth. *Pseudomonas flourescence* (BA-1) followed by *T. harzianum* (Th-5) significantly increased fresh and dry weight compared with untreated control (Fig. 1).

Table (3): *In vivo* screening of selected antagonistic isolates against three pathogenic fungi of pepper and their effect on seedling dry weight.

Isolates	Infested Soil						Non Infested soil	
	F. oxysporum		*P. aphandermatum*		*R. solani*			
	Survived Seedlings % [d]	Dry weight [b]	Survived Seedlings % [d]	Dry weight [b]	Survived Seedlings % [d]	Dry weight [b]	Survived Seedlings % [d]	Dry weight [b]
Known Bioagents:								
Th5	73	14	81	13	80	14	84	14
Pf1	64	10	58	11	62	12	79	11
K1	71	12	74	12	61	10	82	14
Bs1	44	6	49	9	43	9	77	11
Field Isolates :								
Fungi :								
Al-25	76	14	72	12	69	12	87	14
P-10	72	12	67	9	61	9	72	13
P-12	73	7	57	10	62	8	82	13
GA-7	74	13	71	12	70	11	85	13
Bacteria:								
Al-2	56	10	42	11	39	9	77	12
Al-6	44	9	49	9	43	9	52	13
Al-9	56	11	29	9	51	10	73	11
Al-19	47	8	50	11	56	10	77	13
BA1	80	15	76	14	72	12	85	15
BA7	64	10	58	11	62	12	67	11
BA11	69	7	47	9	34	10	77	14
BA15	54	8	52	12	57	10	78	13
Mu-3	76	13	72	14	79	11	82	13
Mu-6	59	12	45	11	49	10	77	13
Ze-1	62	10	53	11	52	10	76	14
Ze-5	42	9	30	10	35	9	74	12
Ze-9	55	11	49	9	35	10	72	13
Ze-13	62	11	52	10	57	10	65	12
Ze-14	72	8	44	6	41	6	78	10
Rizolex.T	86	14	87	13	87	14	77	14
Untreate	50	6	49	6	35	8.	74	12

i) **Treated seeds were sown over water agar culture of each tested. Pathogen and covered with sterilized beatmoss- vermiculite mixture, sprayed with sterilized distilled water and kept at room temperature.**

ii) ***Dry weight of seedling / mg.***

iii) **Seeds were treated with Rizolex –T at rate of 2 g / kg seeds.**

iv) **Survival seedlings (%) was assessed after 25 days of sowing.**

Table (4): **Effect of six antagonistic isolates on incidence of root rot and wilt of pepper plants grown under greenhouse conditions[a].**

Bioagents[b]	Root rot incidence[e]		Fusarium wilt[e]		
	P. aphander-matum	*R. solani*	Incidence (%)	Internal root[f] browning	Foliar wilt rating[g]
Fungi					
T. harzianum (Th-5)	22.2E[h]	22.2B	1.1E	0.7F	0D
Penicillium sp. (GA-7)	44.4C	66.7A	44.4C	2.3D	1C
Penicillium sp. (AL-25)	88.8A	77.8A	66.6A	4.4B	2B
Bacteria					
P. flourescens (BA-1)	11.1E	22.2B	22.2D	1.2E	1.3C
B. cepacia (K1)	66.6B	66.6A	55.6B	2.7C	1.7B
Bacillus sp. (MU-3)	33.3D	66.6A	44.4C	1.1E	1 C
Rizolex-T[c]	33.3D	11.1C	44.4C	2.6CD	1.7B
Untreated [d]	88.9A	88.9A	66.6	5.3A	4.1A

i) Plants (6 week old) were transplanted in pots containing pathogen infested soil.
b) Seedling were treated with spore suspension ($2x10^6$/ml) or bacteria suspension (16^8 cfu/ml)
c) Seedlings were treated with equous solution (500 µg active ingredient/ml).
d) Seedlings were treated with water only.
e) Data were recorded after 45 days of transplanting.
f) Measured as length in cm.
g) Based on a scale of 0= healthy to 5= plant deathas dscribed by Gao (1999).
h) Values followed by the same letter in each column don't differ significantly at $P \leq 0.05$ according to Duncan's multiple range tests.

Efficncy of antagonists in the field

Data in Table (5) indicate that *Trichoderma harzianum* (Th-5) and *Pseudomonas fluorescens* (BA-1) were highly effective for controlling wilt and root rot diseases under field conditions. *Trichoderma harzianum* (Th-5) reduced root rot incidence caused by *P. aphanidermatum* and *Rhizoctonia solani* by 47% and 52% respectively and reduced Fusarium wilt by 37%. Meanwhile, the fungicide Rizolex-T was the best treatments in reducing Rhizoctonia root rot. *Pseudomonas fluorescens* (BA-1) was the most effective treatment in reducing wilt incidence and also greatly reduced internal root browning and foliar wilt rating.

Table (5): Effect of two selected bioagents on incidence of root rot and wilt of pepper plants grown in the field[a]

Bioagents[b]	Root rot incidence[e]		Fusarium wilt[e]		
	P. aphander-matum	*R. solani*	Incidence (%)	Internal root[f] browning	Foliar wilt rating[g]
T. harzianum (Th-5)	13.3B[d]	16.6C	21.3B	8.7B	1.5B
P. flourescens (BA-1)	21.3A	22.3B	16.0C	2.1D	0.0C
Tizolex-T[c]	15.3C	10 D	19.3B	3.0C	0.6B
Untreated [d]	25.3A	34.7A	34.0A	13.6A	3.8A

a) A field experiment was established at Mariot Experimental Station during summer season 2000; 6-weeks old pepper plants were infested with each pathogen before transplanting.
b) Seedling were treated with spore suspension ($2x10^6$/ml) for *T. harzianum* or (16^8 cfu/ml) for *P. flourescens*, or 500 µg active ingredient/ml for Rizolex-T, or water for untreated control, applied 12 h. before transplanting.
c) Data were recorded after 45 days of transplanting.
d) Values followed by the same letter in each column don't differ signficantly at $P \leq 0.05$ according to Duncan's multiple range test.

Data in Fig. (1) also indicate that no significant effect for both bioagents *T. harzianum* and *P. fluorescens* or fungicide Rizolex-T on fresh and dry weight of pepper plants grown in non infested soil. Data in Fig. (2) indicate that fruit yield of plants grown in non infested soil was higher than those of plants in infested soil. However, treatments with either *T. harzianum* and *P. fluorescens* or fungicide Rizolex-T led to increase of fruit yield significantly compared with non treated control.

Effect on pathogen's population :

Total counts of the three pathogenic fungi *Fusarium oxysporum*, *Pythium aphanidermatum* and *Rhizoctonia solani* colonies were determined in rhizosphere of pepper plants which treated with *T. harzianum* (Th-5) and *P. fluorescens* (BA-1) after 15, 30 and 45 days from transplanting. Data in Fig. (3) show that, total counts of *P. aphandermatum* and *R. solani* were decreased in both bioagents treatments after 15, 30 and 45 days from transplanting. *T. harzianum* showed the highest effect to reduce the counts of *P. aphanidermatum* and *R. solani* compared with non-treated control (Fig. 3). Population of *F. oxysporum* was higher after 15 days from transplanting, while *R. solani* and *P. aphanidermatum* gradually decreased in non treated soil. However, *P. fluorescens* treatment reduced counts of *Fusarium oxysporum* compared with *T. harzianum* treatment. Data also indicated that, population of these pathogens did not disappear after 45 days from transplanting of pepper.

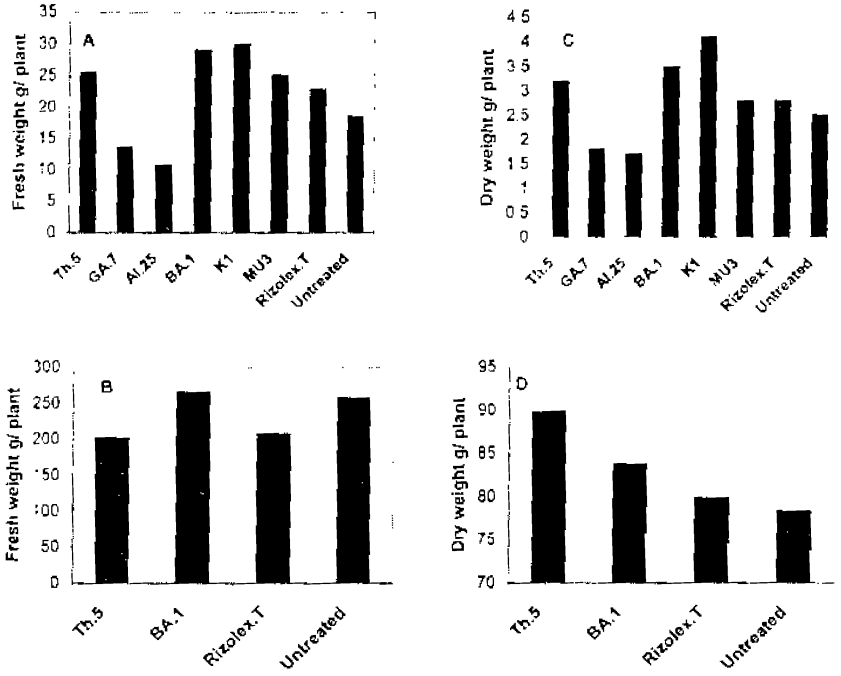
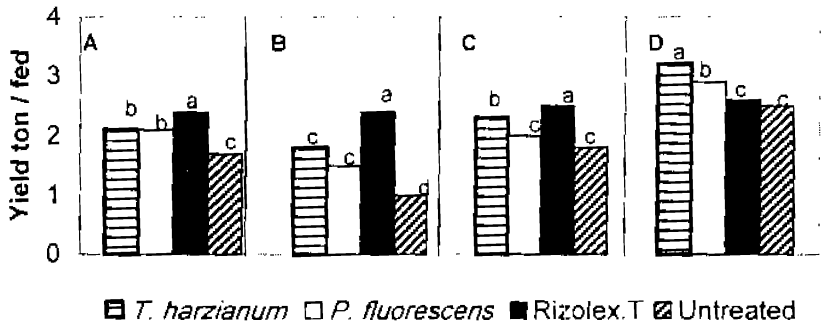
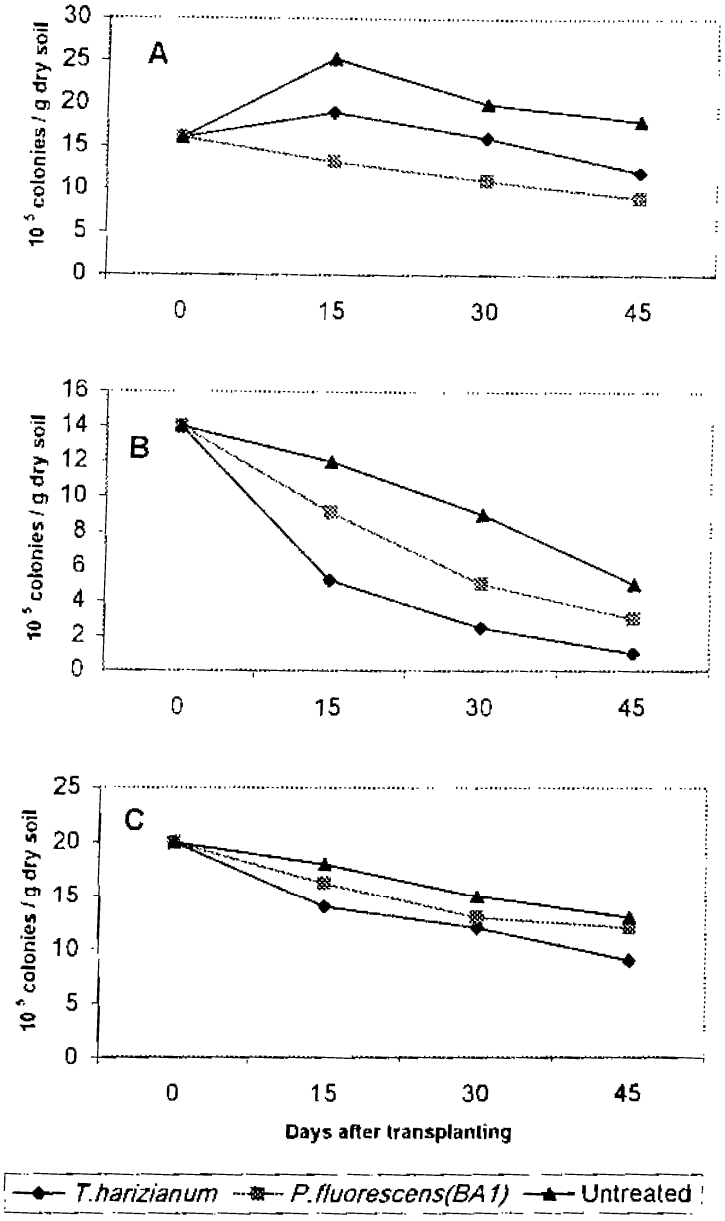


Fig (1) Effect of bioagents treatments on fresh weight (A&B) and dry weight (C & D) of pepper plants grown in pathgen- noninfested soil (A & C) pot experiment , (B & D) in the field.



Fig(2): Effect of treatment of pepper transplants with *Trichoderma harzianum* (Th5) and *Pseudomonas fluorescens* (BA1) on fruit yield in soil infested with *Fusarium oxysporum*(A),*Pythium aphanidermatum*(B) or *Rhizoctonia solani* (C) or non-infested soil (D) under field conditions at Manout Experimental Station (DRC) Coulmn heded by the same latter don't differ significantly at P = 0.05 according to Duncan's multiple range test.



Fig(3):Effect of treatment of pepper transplants with *Trichoderma harzianum* (Th5) or *Pseudomonas fluorescens* (BA1)on soil population density of pepper root rot and wilt pathogens(A):*F.oxysporum*,(B) *P.aphanidermatum*,(C) *R.solani* after 15,30 and 45 days from transplanting in the field.

DISCUSSION

Many bacterial and fungal isolates, collected from rhizosphere of different plants grown in a reclaimed area (Mariout), showed antagonistic effect against three soil borne pathogens of pepper. Host plant had clear effect type on the population of rhizosphere-colonizing microorganisms. High population count of bacteria was recorded with alfaafa, barley and maize rhizosphere, respectively. It appears that soil factors played a minor role in controlling microorganisms biodiversity in this study because all plants were collected from only one soil claimatic zone. These results are in agreement with Germida *et al.* (1997) and Larkin and Fravel (1998). On the contrary, soil factors play a large role in determining fluorescent pseudomonads in tomato rhizosphere (Latour *et al.*, 1996). This suggests that the relative influence of soil and plant factors on biodervsity of microorganisms might be dependent upon the plant species being investigated. The highest number of rhizobacteria might have the potential to reduce survival of certain soil bornee plant pathogens. Attempts to find superior biocontrol microorganisms through isolation of pepper root-colonizing microorganisms were not successful in this study. Although several isolates significantly reduced pathogens growth in *in vitro* and *in vivo* assays, non of isolates of pepper tested in this study had antagonistic efficacy. However, this does not suggest that this approach does not have merit. Ideally organisms should be isolated from plants grown in fields where both pepper plants and the pathogens have been coexisting for several years (Cook, 1993). However, root rot and wilt pathogens of pepper might not be present in the studied fields where different other plants had been grown.

Approximately 20% of the obtained isolates showed activity against growth of pepper root pathogens, *in vitro*. Based on *in vivo* assay, antagonistic isolates which reduced seedling emergence, limited plant growth or increased seedling damping-off were eliminated. Following the final greenhouse experiments, two obvious candidates emerged as biological control agents. *Pseudomonas fluorescens* (PA1) and *T. harzianum* (Th5) were the most effective antagonists, consistently reduced root-rot caused by *R. solani* and *P. aphanidermatum* and wilt incidence relative to non treated controls. *P. fluorescens* isolate (BA1) was originally isolated from barley rhizosphere while *T. harzianum* (Th5), was isolated from wheat. This suggests that the mechanism of action is not host-specific. Both bioagents significantly reduced root rot and wilt of pepper grown under field conditions. Many investigators described biocontrol organisms including *Trichoderma* spp. and fluorescent pseudomonads also demonstrated varying degree of efficacy in reducing pepper root rot (Attia and Abada, 1994, Sid Ahmed *et al.* 1999; Lee *et al*, 1999 and Sharifi-Tehrani and Omati, 1999) and wilt (Abd El-Kader, 1999).

The results indicated that, there was no correlation between *in vitro* antagonistic effect and disease suppression *in vivo*. Only two isolates were most effective antagonists and reduced disease incidence significantly. Thus, ultimate selection of biocontrol agents should based on tests in which

conditions are close to those present in the field. However, the *in vivo* screening assay developed by Mosa *et al* (1997) was relatively fruitful. The value of this technique lies in its ability to substitute for preliminary mass *screening of isolates in greenhouse with consequent savings of space and* times.

Trichoderma harzianum (Th5) and *P. fluorescens* (BA1) have increased plant growth and fruit yield, significantly in the absence of the pathogens. Promotion of plant growth by rhizobacteria and other fungi was also reported by several other invistigators (Harris *et al.* 1994 and Sid Ahmed *et al.* (1999). Several possible mechanisms have been suggested to explain this phenomenon including: production of plant hormones and vitamins, conversion on nonutilizable material into a form that can be utilized by the plant, and increased uptake and translocation of mineral (Windham *et al*, 1986 and Kleifeld and Chet, 1992). Inbar *et al.* (1994) concluded that production of vigorous pepper seedlings, as a result of *T. harzianum* treatment, which are more resistant to soil borne pathogens is advantageous to the producer as well as to the farmer. Application of beneficial microorganisms to the propagative mixture during seedling production in the nursery makes the use of such microorganisms for both plant growth enhancement and biological control more feasible.

A gradual decrease of pathogens population density, as a result of biocontrol treatment, were demonstrated in this study. Various mechanisms was reported to underlay antagonism of *Trichoderma* spp. which included competition, antibiosis and hyperparasitism (Papavizas and Lumsden, 1980 and Tronsmo and Hjeijord, 1998). The ability of antagonistic rhizobacterial isolates to inhibit growth of pathogen, *in vitro*, and to produce certain secondary metabolities has been reported to be important for biological control (Defago and Hass, 1990; Maurhofer *et al.* 1995). Antibiosis is well documented for *P. fluorescens* (Pf5) (Howell and Stipanovic, 1979). Evidence has been also obtained for involvement of siderophores in pathogen suppression (Leong, 1986). However, several biocontrol agents such as *Pseudomonas* spp. (Lius *et al.* 1995; Wei *et al*, 1996) and *Trichoderma harzianum* (Yedidia *et al*, 1999; Sid Ahmed *et al*, 2000) have been used to induce resistance in plants. Further studies are needed to determine the specific mechanisms, interactions and requirements responsible for effective biological control by Th5 and BA1.

In conclusion, *T. harzianum* Th5 and *P. fluorescns* (BA1) from Egyptian soil were effective in suppression of pepper root rot caused by *R. solani* and *P. aphanidermature* and also *Fusarium* wilt either in greenhouse or field tests. Both isolates promoted growth of pepper plants and significantly increased fruit yield. Additional work is necessary in order to demonstrate the biocontrol efficacy of these isolates under commercial greenhouses conditions as a component of integrated control strategy of root rot and wilt diseases of pepper.

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المكافحة الحيوية لأمراض أعفان الجذور والذبول فى الفلفل

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يصاب الفلفل فى مناطق نظم الزراعة المختلفة بأمراض أعفان الجذور والذبول التى تسبب خسائر شديدة فى المحصول ، ونظرا لتباين المسببات المرضية المختلفة المسببة لتلك الأمراض فى حساسيتها للمبيدات الفطرية المستخدمة فى المكافحة وعدم جدواها أحيانا بالاضافة للتأثيرات البيئية الناشئة عن استخدامها فقد هدف هذا البحث الى دراسة امكانية المكافحة الحيوية لتلك الممرضات . تم عزل ٩١ عزلة بكتيرية وفطرية من تربة ريزوسفير نباتات الشعير ، الذرة ، الثوم ، البرسيم الحجازى ، الخردل والفلفل نامية فى حقول بمنطقة مريوط بالساحل الشمالى (مزرعة مركز بحوث الصحراء) كذلك تم الحصول على ٤ عزلات مكافحة حيوية معروفة ، حيث اختبر التأثير التضادى لهذه العزلات تجاه فطريات الفيوزاريوم أوكسيسبورم ، بيثيم أفانديرماتم وريزوكتونيا سولانى على أطباق الأجار . أظهرت ثلاثة وعشرون عزلة فقط تأثيرا تضاديا سواء بدرجة متوسطة أو كبيرة أو تطفلا علويا لميسليوم أى من تلك الممرضات . تم عمل تقييم حيوى لتلك العزلات باستخدام طريقة أطباق التربة لدراسة تأثيرها كمعاملة بذور فى اختزال موت البادرات الناشىء من تلف الممرضات وحيث تم انتقاء أفضل ستة عزلات مضادة أظهرت تأثيرا معنويا فى خفض الاصابة وكذلك زيادة نمو البادرات الناتجة من بذور معاملة : تربة غير معقمة بالممرض تحت ظروف الصوبة . وكانت أفضل العزلات هى بكتريا سيدوموناس فلورسينس BA1 حيث اختزلت حدوث مرض عفن الجذور الناشىء عن فطر البيثيم بنسبة ٨٧% والناشىء عن الريزكتونيا بنسبة ٧٥% وكذلك الذبول الفيوزاريومى بنسبة ٧٥% . بينما اختزلت المعاملة تريكودرما هارزيانم Th-1 بعد عفن الجذور البيثيومى أو الريزوكتونى بنسبة ٧٥% وكذلك الذبول الفيوزاريومى بنسبة ٨٧,٥% . أدت المعاملة بـ BA1 و Th5 الى زيادة الوزن الغض والجاف للنباتات المعاملة والمزروعة فى أصص خالية من الممرض . تم دراسة تطبيق استخدام العزلتان BA1, Th5 تحت ظروف الحقل فى تجربة أجريت بمزرعة مريوط بالموسم الصيفى لعام ٢٠٠٠ وحيث إختزلت كلا العزلتين من حدوث أعفان الجذور والذبول بدرجة معنوية وكذلك أدت المعاملة الى زيادة كبيرة فى محصول الفلفل . ومن ثم فإن الدراسة تؤكد إمكانية تطبيق إستخدام عزلتى تريكوديرما هارزيانم Th-5 ، سيدوموناس فلورسينس BA1 كأحد العوامل فى برنامج المكافحة المتكاملة لأمراض أعفان الجذور والذبول فى الفلفل فى مصر تحت نظم الزراعة المختلفة . إلا انه مازالت هناك حاجة لمزيد من الدراسة لمعرفة مدى تأقلم تلك العزلات الحيوية على أنواع التربة المختلفة وحيويتها وبقائها .