STUDIES ON CERTAIN ROOT-ROT DISEASES INFECTING SOME PLANTS OF FAMILY SOLANACEAE

By

Sahar Metwally Hassan Hamoud
B.Sc. (Agric), Tanta Univ., ARE, 1994

Thesis

Submitted in Partial Fulfillment of the Requirements for the Degree

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Master of Science

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Plant Pathology

Agricultural Botany Department
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CONTENTS

INTRODUCTION

Some solanaceous crops e.g. tomatoes (*Lycopersicon esculentum*) and pepper (*Capsicum annum*) are extremely important vegetable crops in Egypt.

These crop plants are subject to the attack by many soil-borne pathogens causing seedling damping-off, root rots and wilt symptoms, consequently affect their quantity and quality (**Hartman and Fletcher, 1991 and Abada, 1994**).

Control of soil-borne diseases are conventionally carried out by fungicidal seed treatments (**Ohep *et al.*, 1984; Yehia *et al.*, 1984; Satour *et al.*, 1986 and Benhamou, 1992**).

Recently, non-fungicidal applications for plant fungal diseases is one of the major objectives of the plant pathologists all over the world. For the time being, to avoid hazardous of using chemical control, biological control of plant diseases has attracted the attention of most workers (**Elad *et al.*, 1983; Sivan *et al.*, 1984; Sivan and Chet, 1987; Lumsden *et al.*, 1992; Chambers and Scott 1995; Benhamou and Chet, 1996 and Khalifa 1997**. Therefore, the present study aimed to: Study the

1. Effectiveness of some antagonists in controlling damping-off and root rots of tomato and pepper plants.
2. Effect of culture filtrates of selected antifungal microorganisms.
3. Metabolites produced by certain bioagents.
4. Effect of the tested biocontrol agents on growth of tomato and pepper plants.

REVIEW OF LITERATURE

Several soil-borne fungi attack tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annum*) plants causing damping off, wilt and root-rot diseases. These wide spread diseases are caused by several pathogens included *Fusarium* spp., *Rhizoctonia solani, Sclerotium* spp., *Phytophthora* spp. and *Pythium* spp.

Fusarium spp. differed in their ability to cause pre-or post emergence damping off and induces wilt. *Fusarium oxysporum* f. sp. *lycopersici* is wide spread and destructive soil borne pathogen that may be responsible for severe tomato yield losses all over the world (**Jarvis *et al.*, 1975; Jenkins and Averre, 1983; Brammall and Higgins, 1985; Malathrakis, 1985; Fahim *et al.*, 1986; Ricker, 1987; Favrin *et al.*, 1988; Forsberg, 1989; Jarvis, 1989; Kapoor and Kar, 1989; Brammall and Lynch, 1990; Khalifa, 1991 and Lukyanenko, 1991**).

Kapoor (1987) found that several isolates of *F. oxysporum, F. solani, F. Semitectum, F. chlanydosporum* and *F. moniliforme* isolated from wilted tomato plants were compared for their potential to cause pre- or post emergence mortality and induce wilting in a transplanted crop.

Fusarium oxysporum f. sp. *lycopersici* now attacks other Solanaceae species and members of Leguminaceae, Cucurbitaceae and Chenopodiaceae (**Menzies *et al.*, 1990**).

Other pathogens were reported associated with damping off and root rot of tomato and pepper plants.

Pythium aphanidermatum was a most serious soil-borne fungus causing damping-off of tomato seedlings in Florida (**Sonoda, 1973**) and Canada (**Favrin *et al.,* 1988**).

R. solani is a wide spread soil-borne pathogen causing damping off and collar rot of wide range of vegetable plants including solanaceous crops in different countries (**Alavi *et al.,* 1986; Blancard *et al.,* 1991; Hadwan and Khara, 1992 and Moustafa and Khafagi, 1992**).

Biological control of soil-borne pathogens:

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**).

Numerous references covering the *in vitro* and in vivo antagonism of several bacterial, fungal and actinomycetes genera to soil-borne pathogens were reported (**Howell, 1982; Kim and Roh, 1987; Harrison *et al.,* 1991; Askew and Taing, 1994; Duuff *et al.,* 1995; Benhamou *et al.,* 1996; Mansour, 1997 and Haggag (Wafaa), 1998**).

Antagonistic bacteria have been extensively studied as biocontrol agents effective against soil-borne pathogens. Among 20 genera of bacteria, *Bacillus* spp., *Pseudomonas* spp. and Actinomycetes

(*Streptomyces* spp.) were widely used for their characteristics as biocontrol agents (**Cook and Baker, 1983 and Yuen *et al.*,** 1985).

Bacillus spp. by their abilities to produce spores tolerating severe condition were recommended as biocontrol agents in general and *B. subtilis* in particular appears to be the most effective as a biocontrol agent. *In vitro*, it showed an inhibitory effect on the mycelial growth of plant pathogenic fungi (**Osman *et al.*, 1986; Dhedi *et al.*, 1990 and Phae *et al.*, 1992). Lima and Escobar (1990)** found that *B. subtilis* inhibited germination and growth of *F. equisti* isolated from tomato seedling after 24 hours incubation at 27-30°C. Under field conditions, such bacteria improved plant growth of many plant species in steamed and natural soil, due to decrease incidence of diseases caused by several plant pathogens (**Merriman *et al.*, 1974; Broadbent *et al.*, 1977 and Yuen *et al.*, 1985**).

Loeffer *et al.* (1986) found that, two antifungal antibiotics were produced by *B. subtilis*. One of them was identified as dipeptide compound named bailysin, which inhibited yeast and bacteria, where as the other was identified as fengymycin (a complex of closely related lipopeptide components) showed antibiotic activity to protect plants from the pathogenic action of soil-borne fungi. Also, **Ferreira *et al.* (1991)** indicated that *Bacillus* spp. produced 66 different antibiotic compounds.

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

Phae *et al.* (1992) suggested that in field trials, when rice straw was immersed in a culture suspension of *B. subtilis* and then mixed with soils infested with *F. oxysporum* f. sp. *radicis-lycopersici*, the subsequent occurrence of crown and root-rot of tomato was reduced. Also, *B. subtilis* reduced damping-off caused by *R. solani* and *Pythium aphanidermatum* in cucumber (**Wolk and Sorkar, 1994**) and *F. solani* in cowpea and broad-bean (**Mansour, 1997**).

Several *Pseudomonas* spp. especially *P. fluorescens* have been associated with inhibition of several soil borne diseases. *In vitro* studies, *P. fluorescens* showed antagonistic activity against *R. solani, F. oxysporum* and *Pythium* spp. which caused damping-off and root rot of different crops (**Howell and Stipanovic, 1980; Alabouvette, 1990 and Wolk and Sorkar, 1994**). Under field conditions, *P. fluorescens* reduced disease incidence caused by *R. solani* to 40-70% when applied to soil at 10^6 propagates/g of soil. The suppressive effect was more evident in steam-sterilized soil than non sterilized field soil (**Kim and Roh, 1987**). It has been reported that such bacteria reduced cucumber wilt incidence to 50% than in untreated plots. Also, *P. fluorescens* controlled root-rot disease caused by *R. solani* and *Pythium* spp. in cotton (**Park, 1990; Park *et al.* 1991; Hagendorn and Bardinelli, 1993 and Cartwright and Benson, 1995**) and several soil-borne pathogens in the field and greenhouse (**Benhamou *et al.,* 1996 and Haggage (Wafaa), 1998**).

Several antibiotics were produced by *Pseudomonas* spp. which inhibited growth of many soil-borne fungi. Among these antibiotics, hemipyocianine chlorofraphin, phenazine 1- carboxylic acid, pyrrolinitrin, pyoluteonin and pseudane which were produced by 21

Pseudomonas strains (**Hasegawa *et al.*, 1990**). In addition to **Harrison *et al.* (1991)** reported that pseudomycins is a family of novel peptides isolated from *P. syringae* processing broad-spectrum antifungal activity against a broad range of plant pathogenic fungi.

Presence and role of actinomycetes in the rhizosphere have been widely studied and their role as biocontrol agents of soil-borne fungal diseases was mentioned by **Saracchi *et al.* (1992) and Dormann (1993)**. They reported that *Streptomyces* inhibited growth of *Fusarium* spp., *R. solani in vitro*. In greenhouse trials Streptomyces S 57 inhibited 13 out 18 tested fungal species. Application of *Streptomyces* spp. showed a significant reduction of root rot disease caused by *R. solani* and *Fusarium* spp. of tomato (**Shahida-Parveen, *et al.* 1991**).

Numerous fungi have been documented as effective antagonists against several important soil-borne pathogens *Trichoderma* spp., *Gliocladium* spp., *Penicilium* spp. have been most studied in the biocontrol of root pathogens. Antagonistic *Trichoderma* spp. are regarded as being of special interest for use as biocontrol agents and succeeded to control soil-borne diseases (**Harman *et al.*, 1980; Tu, 1980; Lumsden and Lock, 1989; Papavizas, 1985 and Adams, 1989**).

Recently, screening studies *in vitro* showed that *Trichoderma* spp. had high antagonistic effect against root-rot pathogens. It attacked the host by hyphal coils, hooks or appressoria. Lysed sites and penetration holes were found in hypha of the plant pathogenic fungi. Excreted lytic extra cellular B (1-3) gluconase and chitinase into the growth medium and even into the soil (**Elad *et al.*, 1980, 1981, 1982 and 1983; Datnoff *et al.*, 1995 and Lo *et al.*, 1996**).

It was reported that *Trichoderma* spp. and *Gliocladium virens* showed strong antagonistic activity to *F. oxysporum* f. sp. *lycopersici* and *Phytophthora cinnamomi* by mycoparasitism and over growth of the pathogens (**Cipriano *et al.*, 1989 and Chambers and Scott, 1995**).

Antibiosis is potentially a principal component of mechanism of the biocontrol by *Trichoderma* spp. and *Gliocladium* spp., *G. virens* produced an array of metabolites were identified as antifungal and antibacterial compounds, i.e. viridin, sesquiterfen, gliotoxin, gliovirin, gliocladic acid, heptelidic acid (avocetin), viridiol and valinotricin. Gliotoxin specifically has been implicated in biocontrol mechanism, in addition to that suzukacillin and alamicine are peptide antibiotics with antifungal and antibacterial properties. Dermadin is an unsaturated monobasic acid, active against gram negative and gram positive bacteria and a wide range of pathogenic fungi (**Abd El-Moity, 1981 and Smith *et al.*, 1990**).

MATERIAL AND METHODS

1. Survey, isolation and identification of tomato and pepper soil-borne pathogens:

Diseased tomato and pepper plants at different stages of growth showing various degrees of root-rot, stem rot and wilt symptoms were collected from different Governorates, i.e., Kafr El-Sheikh, Gharbiya Dakahliya and Behira during 1995-1996 seasons. Diseased roots and stem bases were carefully washed by running tap water to remove adhering soil particles, cut into small pieces and surface sterilized by dipping in 3% sodium hypochlorite for 3 minutes, then rewashed with sterilized water several times and finally dried between two sterilized filter papers. Pieces were planted in Petri dishes containing potato dextrose agar medium (PDA). Plates were incubated for 3-6 days at 28°C and examined daily to check up developing fungal growth. Fungi that grew on such medium were purified through hyphal tip. The fungal cultures were maintained on PDA medium for further studies.

2. Pathogenicity test:

The isolated fungi from tomato plants were tested for their pathogenicity on the Castel Rock tomato cultivar, while those isolated from pepper plants were tested on California wonder cultivar.

Seeds of tomato and pepper plants were obtained from Dept. of Horticulture, Agricultural Research Center, Giza, Egypt. Pathogenicity tests were conducted in the greenhouse at the Faculty of Agriculture Kafr El-Sheikh, Tanta University.

2.1. Preparation of pathogenic inocula and soil infestation:

Pathogenic inocula of all isolated pathogens were prepared on corn meal sand medium (95 gm clean moistened sand and 5 gm corn meal) (**Sneh *et al.,* 1991**). Medium was placed in glass bottles of 500 ml capacity and autoclaved for 30 minutes at 1.5 air pressure, inoculated using agar discs (6 mm diameter) obtained from the periphery of 7 days old colony of each isolated fungi, then incubated at 28°C for 15 days for infesting soil. Sterilized pots (25 cm diameter) filled with autoclaved clay loam soil were inoculated with each of the fungal isolates at the rate of 3% w:w of soil.

The infested soil was moistened thoroughly every other day for one week. In the check pots, the soil was mixed with the same amount of sterilized corn meal sand medium. Pots were sown by tomato seeds and pepper seeds (5 seeds/pot). The seeds were surface sterilized with 3% sodium hypochlorite for 3 minutes, then they were washed several times with sterilized water. The pots were watered periodically. Three replicates were used for each treatment which were arranged in completely randomized design.

The identity of the pathogenic isolates was carried out at the Department of Agric. Botany, Fac. of Agric., Kafr El-Sheikh, Tanta Univ. and Confirmed at Mycology Laboratory, Plant Pathology Institute, Agricultural Research Center, Giza, Egypt.

2.2. Disease assessment:

Disease assessment was recorded as percentage of pre-emergence damping-off after 15 days of sowing, post-emergence damping off, root-rot and wilt symptoms were recorded up to 90 days as described by

Khalifa, (1987). Pre and post emergence damping-off were estimated as follows:

$$\% \text{ Pre emergence damping off} = \frac{\text{No. of non emerged seeds}}{\text{No. of sown seeds}} \times 100$$

$$\% \text{ Post emergence damping off} = \frac{\text{No. of killed seedings}}{\text{Total No. of emerged seedlings}} \times 100$$

Survived seedlings were removed, washed and diagnosed, they were scored for *R. solani* on (0-3) scale described by **(O'Sullivan and Kavanagh, 1991)** as follows:

0 = No necrosis

1 = Slight necrotic lesions on the root or hypocotyls.

2 = Lesions extending around the hypocotyls

3 = Seedling killed by disease (or not emerged seeds).

A disease index for each assessment was expressed as a percentage of maximum possible infection as follows:

$$\text{Disease index} = \frac{100\,(X + 2Y + 3Z)}{3\,(W + X + Y + Z)}$$

Where:

W = Seedlings in class 0

X = Seedlings in class 1

Y = Seedlings in class 2

Z = Seedlings in class 3

For the assessment of Fusarium wilt or root rot, survived plants were removed 45-90 days after sowing. They were scored for disease on 0-5 scale described by **Kraft and Papavizas, (1983)**. Where:

0 = Healthy plant (no infection).

1 = Very weak infection (tiny discoloration covering 10% of root surface area).

2 = Weak infection (tiny necrotic lesions covering 11-25% of root surface area).

3 = Medium size lesions with corky tissues covering 26-50 of root surface area.

4 = Sever infection (necrotic lesion covering 51-75% of root surface area).

5 = Very sever infection (complete death of plant).

Disease severity was expressed as a weight average of the disease index per pot. This was calculated by the following equation:

$$\text{Disease severity} = \frac{\sum \text{(disease index x number of seedlings)}}{\text{Total number of seedlings}}$$

3. Screening for biocontrol agent:

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, then 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. Portions 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 (**Waksman, 1957 and King *et al.*, 1954**).

For isolating fungal antagonist (s) .the method recommended by **Elad *et al.* (1980)** was followed.

For isolating Actinomycete(s) starch nitrate agar medium was used (**Waksman, 1957**). After incubation for 24 h. bacteria and

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.

3.1.1. The bacterial antagonist(s):

The bacterial and Actinomycetes antagonists were tested by streaking 2 cm-long on one side of the medium within Petri dish (9 cm in diameter). Pathogen disks (6 mm diameter) were taken from 3-7 days-old cultures were put on the opposite side of the Petri dish.

Plots inoculated with each of the pathogenic fungi only were used as checks. Plates were incubated at 28°C. Three replicates were used for each test. The radius of the 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.*, 1987**) as follows:

Relative power of antibiosis of bacterial antagonists against the pathogenic fungi (R.P.A.) = $\frac{Z}{C}$

Z = Diameter of inhibition zone

C = Diameter of spotted antagonistic isolate.

3.1.2. Identification:

Identification of the isolated bacterial antagonists was performed according to their morphological and physiological properties (**Buchana and Gibbon, 1974 and Bergey's Manual, 1984**). However, identification was confirmed by Department of Bacterial Disease and Biological Control, Plant Pathology Institute, Agriculture Research Center, Giza, Egypt.

3.2. Fungal antagonist (s):

Potato dextrose agar (PDA) plates were inoculated with a disc of each of the isolated pathogenic fungi (6 mm diameter) from 3-7 days old culture. Opposite to the pathogenic fungus, a disc of 3-7 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 seven days.

Degree of antagonistic effect was scored according the scale adopted by **Bell *et al.*, 1982**.

Where:

Class 1	=	Fungal antagonist completely over grew the pathogen and covered the entire medium surface.
Class 2	=	Fungal antagonist over grew at least two-thirds of the medium surface.
Class 3	=	Fungal antagonist and the pathogen each colonized approximately one half of the medium and neither of two organisms appeared to dominate the other.
Class 4	=	The pathogen colonized at least two-third of the medium surface.
Class 5	=	The pathogen completely overgrew the antagonist and covered the entire medium surface.

3.2.1. Identification:

The bioagent microorganisms were identified according to **Gilman (1957) and Rifai (1969)** at the Dept. of Agric. Botany, Faculty of Agriculture, Kafr El-Sheikh as well as Department of Bacterial Disease and Biological Control, Plant Pathology Institute, Agriculture Research Center, Giza, Egypt.

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

Different liquid media i.e., PD amended with 0.2% yeast extract was used for fungi, nutrient glucose media and king's broth media were used for bacteria. Each bioagent isolate was inoculated in 250 ml flask contained 50 ml of each medium and incubated at 28°C for 15 and 6 days for fungi and bacteria, respectively with continuos 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 disc (6 mm diameter) obtained from 7 days-old colony of each pathogenic fungal growth. Plates without any culture filtrate were used as a control and incubated at 28°C. Linear growth of the pathogens were measured until the control plate reached the edges. The inhibition percent was calculated using the formula of **Vincent (1927).**

$$\text{Percent of inhibition of fungal growth (I)} = \frac{C-T}{C}$$

Where:

C = Fungal growth of check

T = Fungal growth of treatment.

5. Metabolites produced by certain bioagents:

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

The antibiotic produced by *G. virens* and *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 culture of the bioagents was then centrifuged at 16.000 g for 10 min. The antifungal compound of each was extracted with an equal volume of 80% aqueous acetone and the acetone 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 were assayed for 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 gar (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.

5.2. Total phenols:

Culture supernatant of different antagonistic microorganisms were subjected to calourimetric determination of total phenols using the folin-Denis reagent for phenols with spectrophotometer (**Association of Official Analytical Chemists 1975**).

6. Greenhouse experiments:

6.1. Biological control:

6.1.1. Bacterial antagonists:

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

6.1.1.a. Preparation of bacterial inoculum and inoculation soil:

Inocula of the antagonistic isolates were prepared by growing them on nutrient broth media for *Bacillus subtilis* or on king's broth media for *Pseudomonas fluorescens* or P.D. media for *Actinomycte* sp. in conical flasks (500 ml) at 28°C for 5 days using shaking incubator (100 rpm). Cell suspension was diluted and adjusted to 10^8 CFU/ml of *B. subtilis* and *P. fluorescens* and 10^7 propagules of Actinomycetes.

Soil was inoculated with antagonistic bacterial isolates at concentration of 10^8 cell/gm at time of planting.

6.1.1.b. Design of experimental treatments were:

1. Untreated (control).
2. Treated with each pathogen to be tested only 7 days before planting.
3. Infested soil + suspension of *B. subtilis* 10^8 cell/gm of soil.
4. Infested soil + suspension of *P. fluorescens* 10^8 CFU/gm of soil.
5. Infested soil + suspension of *Actinomycte* sp. 10^7 propagules/g of soil.
6. Infested soil + benomyl (0.1%).

6.1.2. Fungal antagonists:

Different antagonistic fungi were used as suspension or a wheat bran medium to control root rot disease incidence of tomato and pepper seedlings. They were studied in non-sterilized soil in pots.

6.1.2.a. Preparation of fungal inoculum and inoculating soil:

For preparation of inocula of isolates, *T. harzianum* and *G. virens* were grown on PDA medium for 7 days. A wheat bran medium was autoclaved for 1 h. at 121°C on two successive days as described by **Elad *et al.,* 1980**. Autoclaved bottles, contain 200 gm of the medium, were inoculated with 6 mm diameter agar disks of the antagonistic fungal isolates and incubated for 15 days at 28°C. Soil was inoculated with the biotic fungal isolates on the day of planting at the rate of:

1. *T. harzianum*: 3% of soil weight.
2. *G. virens*: 2% of soil weight.

6.1.2.b. Design of experimental treatments were:

1. Untreated (control).
2. Infested soil with each of pathogens 7 days before planting.
3. Infested soil + *T. harzianum* at time of planting.
4. Infested soil + *G. virens* at time of planting.
5. Infested soil + Benomyl (0.1%).
6. Infested soil + wheat brane alone at time of planting.

6.2. Effect of tested biocontrol agents on morphological characteristis of tomato and pepper plants:

The effect of tested biocontrol agents on different morphological characteristics i.e., plant height, average number of leaves, dry weight of shoot and dry weight or root/plant was studied at the end of experiment.

7. 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

1. Survey and isolation of tomato and pepper wilt and root-rot pathogens:

A survey study was carried out during 1995/96 season to detect the main pathogens associated with wilt and root-rot symptoms of tomato and pepper plants.

Isolation trails were carried out from diseased samples collected from different localities at Kafr El-Sheikh, El-Gharbia, El-Dakahliya and El-Behira Governorates.

Isolation of the pathogens was performed from roots at different stages of plant growth resulted in 60 fungal isolates belong to six fungal genera, i.e. *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., *Sclerotium* spp., *Verticillium* spp. and *Alternaria* spp. The occurrence and frequency of fungi associated with diseased samples differed according to the locality from which the samples were collected. The highest number of fungi was isolated from samples collected from Kafr El-Sheikh Governorate (25 isolates) followed by Gharbia (14 isolates), Behira (11 isolates) and Dakahliya (10 isolates).

The prevalence of each fungus was not always the same in the four Governorates (Table 1). *F. oxysporum* was the most dominant at Kafr El-Sheikh, followed by *R. solani* and *Pythium* sp. At Al-Gharbia *R. solani* showed the highest frequency of isolates followed by *F. oxysporum.* At El-Behira, *Sclerotium* spp. was the dominant pathogen while at Dakahlia *Verticillium* spp. showed the highest occurrence followed by *Pythium* spp.

Table (1): Fungi isolated from wilted and root-rotted tomato and pepper plants collected from different Governorates of the Delta during 1995/96 season.

Governorates	No. of isolates	Isolated fungi
Kafr El-Sheikh	8	*Fusarium oxysporum*
	3	*F. moniliforme*
	2	*F. semitectum*
	5	*F. solani*
	4	*Rhizoctonia solani*
	4	*Pythium* spp.
	2	*Alternaria* spp.
El-Gharbia	1	*F. semitectum*
	3	*F. oxysporum*
	2	*F. solani*
	4	*R. solani*
	2	*Alternaria* spp.
	2	*Pythium* spp.
Beheira	2	*F. solani*
	2	*F. oxysporum*
	2	*R. solani*
	3	*Sclerotium* spp.
	2	*Pythium* spp.
Dakahliya	1	*F. solani*
	3	*Verticillium* spp.
	2	*Pythium* spp.
	1	*F. oxysporum*
Total isolates	60	

2. Pathogenicity test:

The pathogenic potentialities of the most frequent isolated fungi i.e. *Fusarium oxysporum* f. sp. *lycopersici*, *Rhizoctonia solani, Fusarium solani* and *Pythium aphanidermatum* were tested under the greenhouse conditions using Castle Rock tomato cultivar and California Wonder pepper cultivar.

Data presented in Table (2) show pre & post emergence damping off as well as disease index of tomato seedlings grown in soil infested with *F. oxysporum* f. sp. *lycopersici* or *R. solani*. Results indicated that *R. solani* was the most aggressive pathogen in inducing pre-emergence damping off of tomato plants (93.33%), while *F. oxysporum* f. sp. *lycopersici* showed the highest percent of post emergence damping off (58.33%).

Data presented in Table (3) show the reaction of California Wonder pepper cv. toward infection with the tested soil-borne fungi. These data show that *Pythium aphanidermatum* followed by *Rhizoctonia solani* caused the highest percent of pre-emergence damping off (93.33 and 86.67%, respectively). On the other hand, percent of post emergence damping off was indicated by *Fusarium solani* (58.89%).

3. Biological control studies:

3.1. 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 phytopathogenic fungi. Each of the selected isolates was tested for purity and designated with a code number. Preliminary examination indicated that 33 of the antagonistic isolates were aerobic and spore forming, whereas 9 isolates were pigment producer, aerobic and short rods and 3 isolates were Actinomycetes.

Table (2): Pathogenicity tests of fungal isolates to Castle Rock tomato cv. under greenhouse condition.

Tested fungi	Disease expressions			
	% pre-emergence damping off	% post emergence damping off	% survival plants	Disease index
Fusarium oxysporum f. sp. *lycopersici*	33.33 b	58.33 a	8.34 b	78.33 b
Rhizoctonia solani	93.33 a	0.00 b	6.67 c	92.11 a
Control (non-infected)	6.67 c	0.00 b	93.33 a	0.00 c
L.S.D.5%	0.94	0.38	-	1.96

Table (3): Pathogenicity tests of fungal isolates to California wonder pepper cv. under greenhouse condition.

Tested fungi	Disease expressions			
	% pre-emergence damping off	% post emergence damping off	% survival plants	Disease index
Fusarium solani	30.00 c	58.89 a	11.11	81.11 c
Rhizoctonia solani	86.67 b	0.00 b	13.33	85.99 b
Pythium aphanidermatum	93.33 a	0.00 b	6.67	92.59 a
Control (non infected)	6.67 d	0.00 b	93.33	0.00 d
L.S.D.5%	2.57	0.94	-	1.60

Identification of *Bacillus* spp. and *Pseudomonas* spp. were carried out using the morphological and physiological properties which presented in Tables (4, 5). Data presented in Table (4) indicated that the identified bacteria was *Bacillus subtilis*. While data presented in Table (5) indicated that the identified bacteria was *Pseudomonas fluorescens*.

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

Data presented in Table (6) show that some of the antagonistic isolates of *B. subtilis* had limited inhibitory spectrum namely isolate no. B_1, B_{27} and B_{78} which inhibited *R. solani*, *F. oxysporum* f. sp. *lycopersici* and *F. solani* respectively. Similarly *P. fluorescens* isolate no. 190 inhibited *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 no. 5, 8, 13, 18, 24, 33, 35, 44 and 51 of *B. subtilis* as well as isolates no. 5 and 35 of *P. fluorescens*. However, isolate no. 5 of *B. subtilis* was the best antagonist among all isolates of such a bacteria.

Also, data presented in Table (6) show that two isolates of *Actinomycetes* spp. had limited inhibitory spectrum namely isolates no. 2 and 3. However, isolate no. 1 of *Actinomycetes* sp. was the best antagonist among all isolates of such microorganism.

Fungal antagonists were isolated from different soil rhizosphere samples of healthy tomato and pepper plants collected from the different surveyed Governorates. More than 200 fungal isolates were tested for their antagonistic effect against the phytopathogenic fungi.

Table (4): Morphological characteristics and biochemical activities of the antagonistic isolate (B_5) identified as *Bacillus subtilis*.

Testes	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 (5): Morphological characteristics and biochemical activities of the antagonistic isolate ($P_{.35}$) identified as *Pseudomonas fluorescens*.

Testes	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

Table (6): Relative power of antibiosis (RPA) of bacterial antagonists against the major 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		
		Fusarium oxysporum f. sp. *lycopersici*	*Rhizoctonia solani*	*Fusarium oxysporum* f. sp. *lycopersici*	*Rhizoctonia solani*	*Pythium aphanidermatum*
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	1.87e-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.

12 isolates out of these isolated fungi exhibited antagonistic effect against one or more of the tested pathogens. These fungal isolates were identified as *Trichoderma harzianum* and other five different *Trichoderma* spp. one isolate of *Gliocladium virens* and other two different Gliocladium isolates, *Paecilomyces* sp., *Myrothium* sp. and *Geotrichum* sp.

Data presented in Table (7) show that isolate *T. harzianum* proved to have the highest effect against the tested phytopathogenic fungi while, T_2, T_4, T_5, T_6 isolates showed moderate effect. T_3 had the least effect against the tested pathogenic fungi. All Trichoderma isolates had their antagonistic effect through their growth over the pathogen (Table 7) and Figs. (1, 2, 3, and 4).

Data presented in Table (8) show the inhibitory effect of *Myrothecium* sp., *G. virens, Gliocladium* spp., *Paecilbmyces* sp. and *Geotrichum* sp. However, *Gliocladium virens* was the best antagonist as shown in Table (8) and Fig. (4).

3.2. Effect of culture filtrates of the different antagonists on mycelial growth of the tested phytopathogenic fungi of tomato and pepper plants:

Filtrates of the antagonists to be tested were examined for their inhibitory action to all the tested phytopathogenic fungi. Different concentrations of each antagonist i.e. 10, 25 and 50% v/v of the media were added to the PDA medium at 45°C then poured in Petri dishes. Each pathogenic fungus disc (6 mm diam.) taken from *F. oxysporum* f. sp. *lycopersici, R. solani, F. solani, R. solani* II and *P. aphanidermatum* old culture was put in the center of the Petri dish. It is clear from the data the culture filtrate of *T. harzianum* had the highest effect which inhibited mycelial growth of the different tested phytopathogenic fungi.

Table (7): Effect of *Trichoderma spp.* against the tested phytopathogenic fungi of tomato and pepper plants.

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

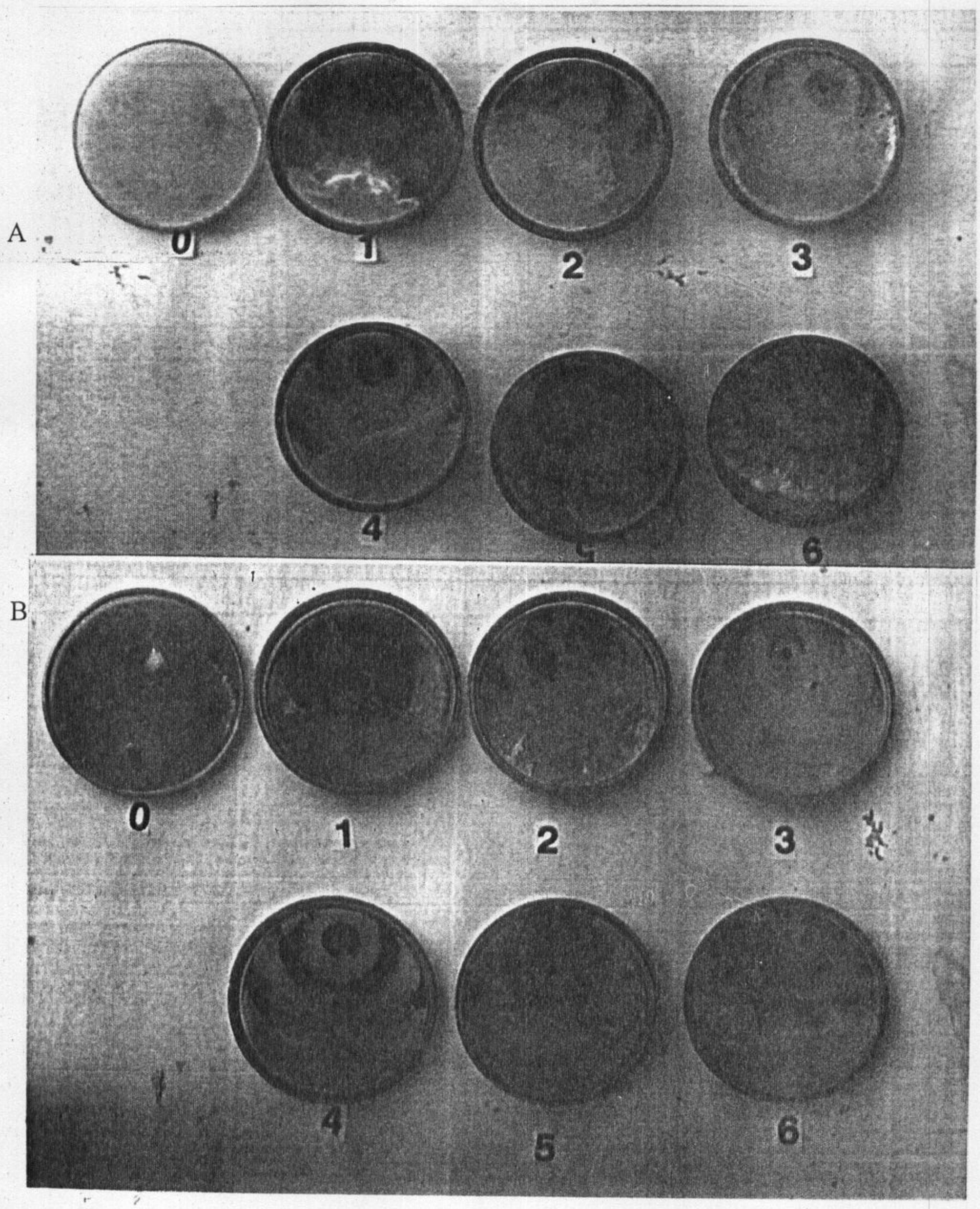


Fig. (1): The antagonistic effect of *Trichoderma spp.* (1-6) against *P. aphanidermatum* (A), *R. solani* (B).

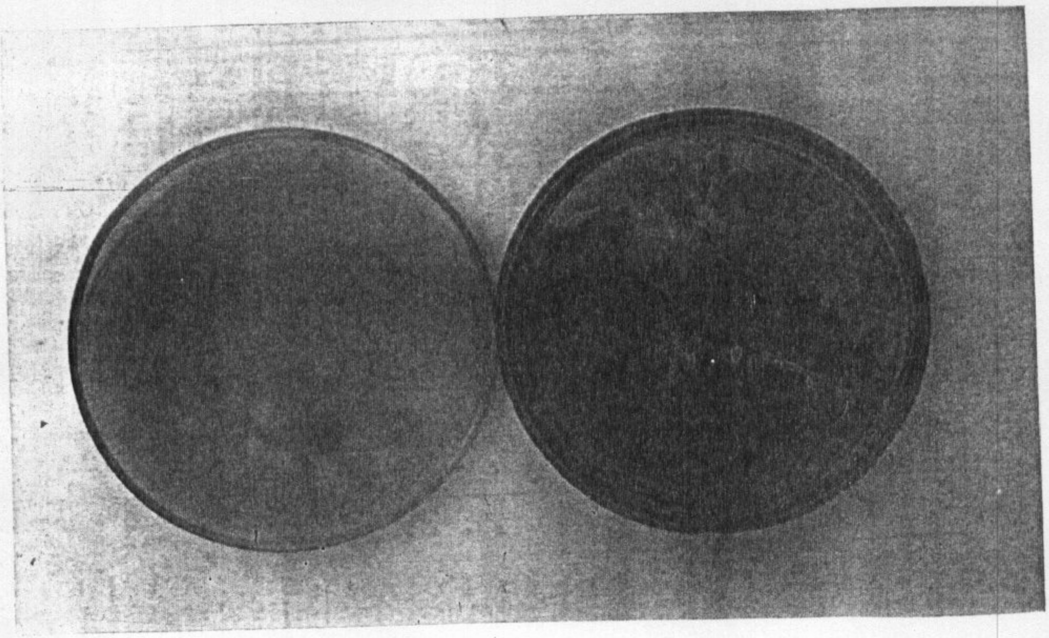


Fig. (2): Effect of *T. harzianum* against *Pythium aphanidermatum*.

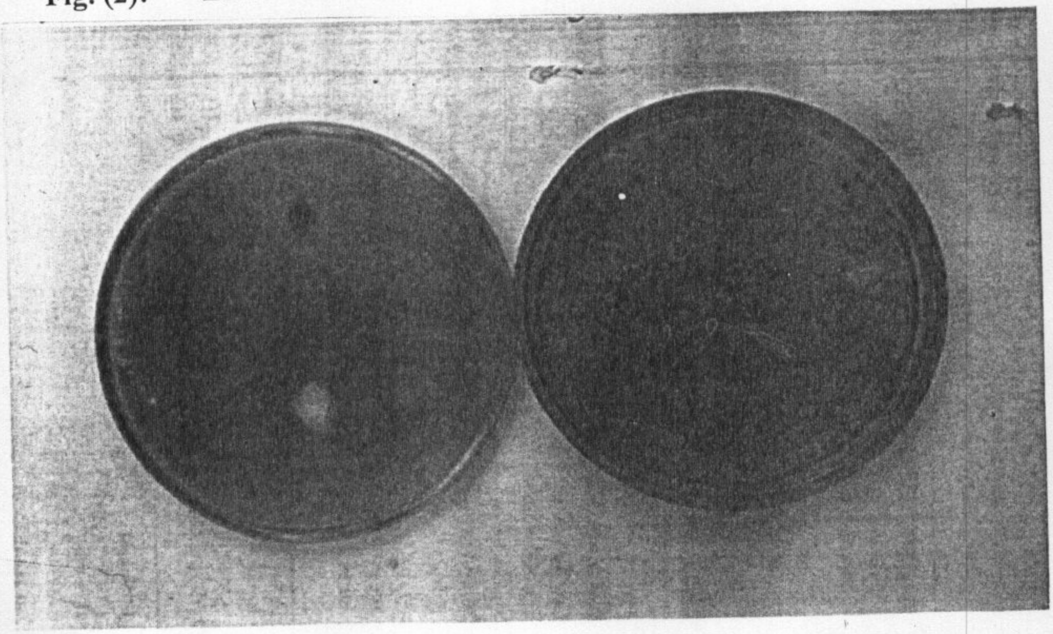


Fig. (3): The antagonistic effect of *T. harzianum* against *Rhizoctonia solani*.

Table (8): Efficiency of other fungal antagonists against the major soil borne fungal pathogens of tomato and pepper plants.

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

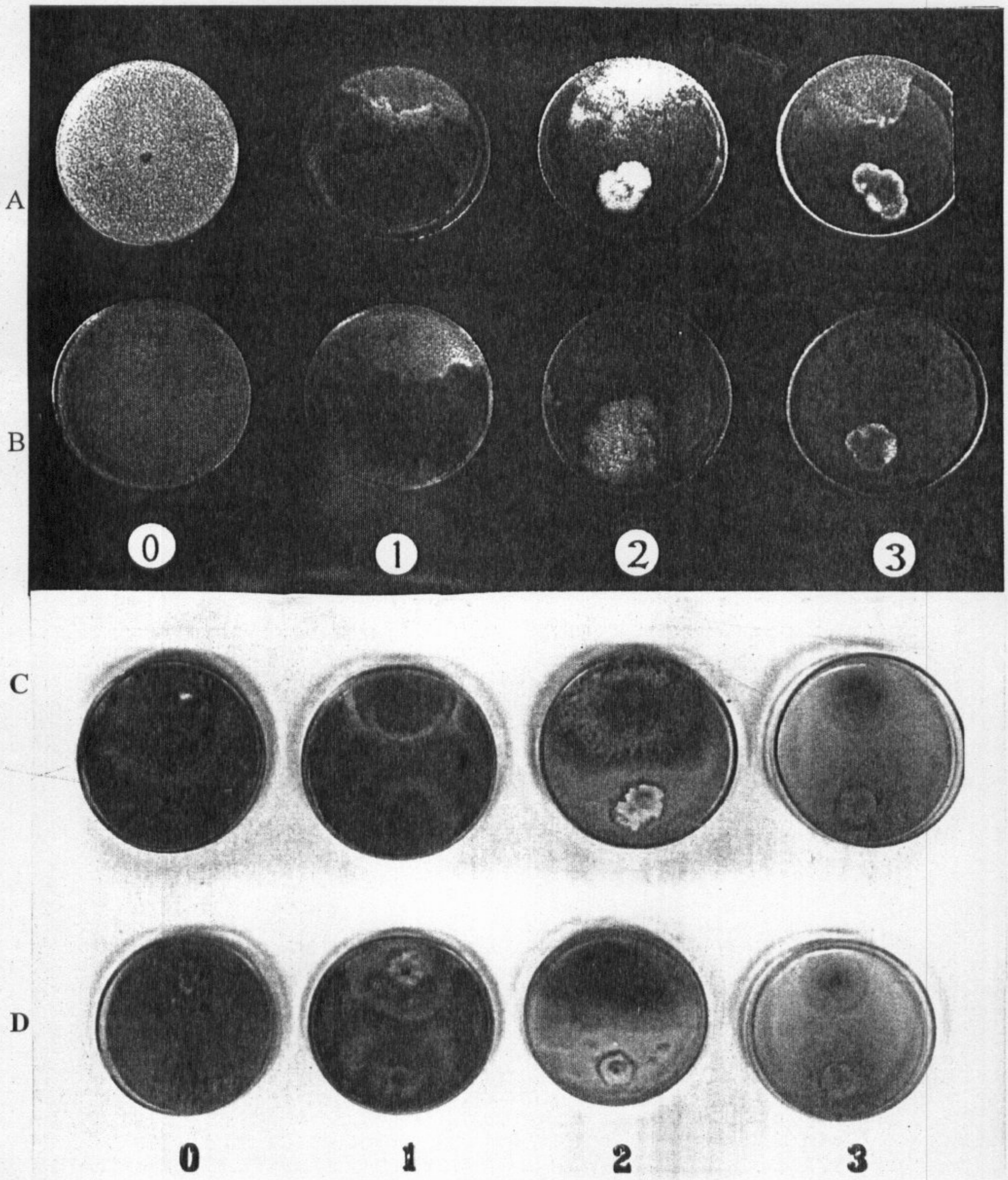
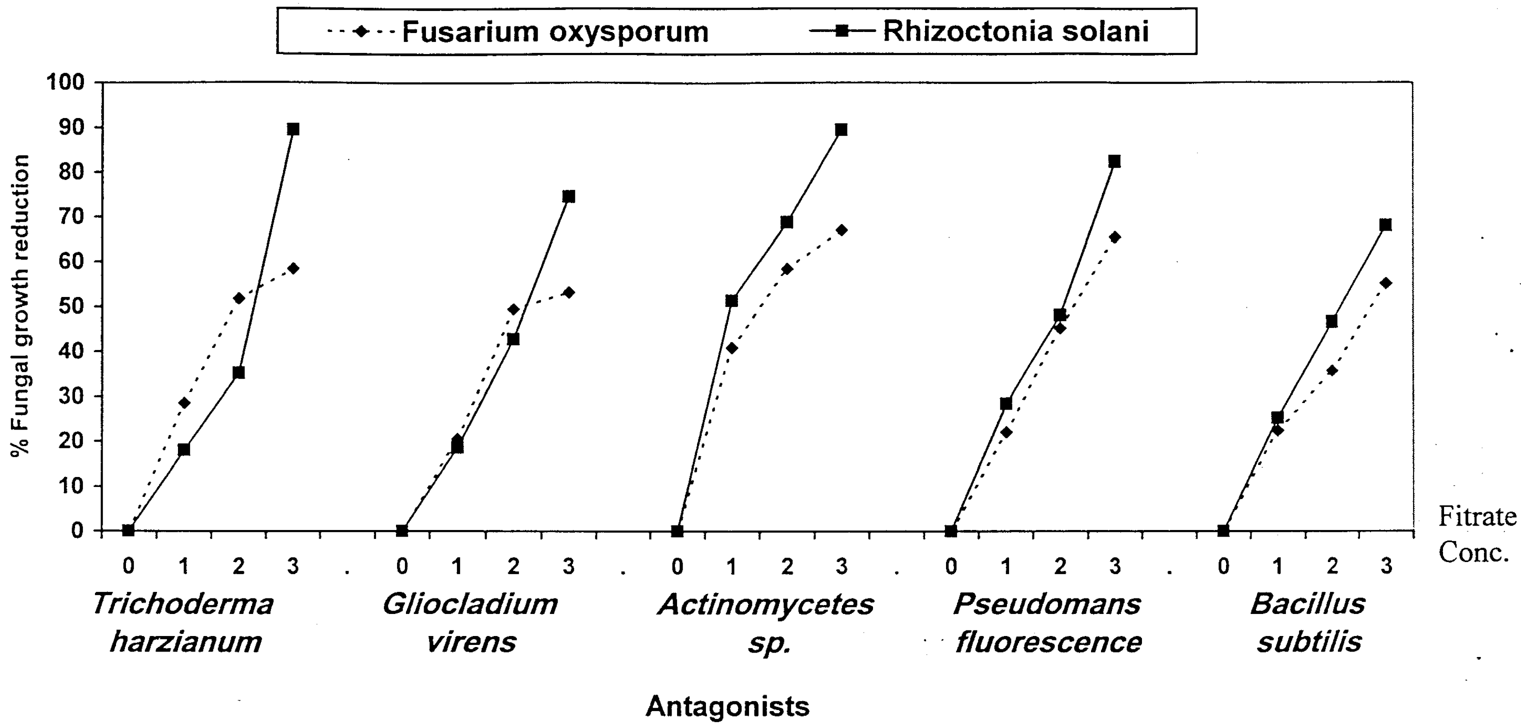


Fig. (4): The antagonistic effect of *T. harzianum* (1), *G. virens* (2), *Actinomycete sp*. (3) against *P. aphanidernatum* (A), *R. solani* (B), *F. oxysporum f. sp. lycopersici* (C), *F. solani* (D)

Table (9): Effect of culture filtrates of different antagonistic microorganisms on the growth reduction percentage of the soil borne fungal pathogens of tomato and pepper plants.

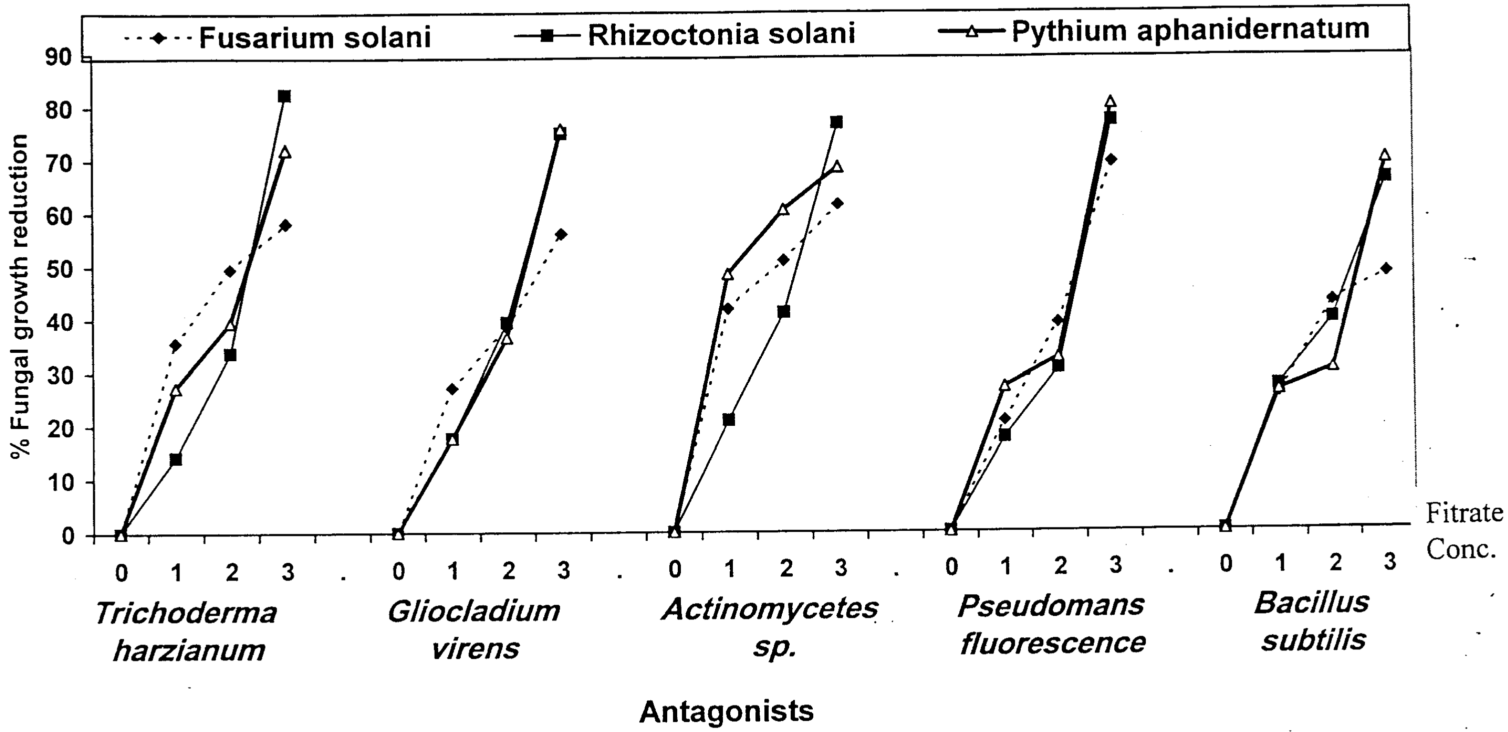
Antagonists	Filtrate conc.	% fungal growth reduction of pathogens of				
		Tomato plants		Pepper plants		
		Fusarium oxysporum f. sp. *lycopersici*	*Rhizoctonia solani*	*Fusarium solani*	*Rhizoctonia solani*	*Pythium aphanidermatum*
Trichoderma harzianum	0	0.00 d	0.00 d	0.00 d	0.00 d	0.00 d
	1	28.54 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
Gliocladium virens	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
Pseudomonas fluorescens	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
Bacillus subtilis	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
Actinomycete sp.	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.



(Tomato)

Fig. 5 : Effect of culture filtrates of different antagonistic microorganisms on the reduction percentage of fungal growth isolated from diseased tomato plants.
(Concentrations of culture filtrates 0 = 0 %., 1=10%, 2 = 25%, 3=50%)



(Pepper)

Fig. 6: Effect of culture filtrates of different antagonistic microorganisms on the reduction percentage of fungal growth isolated from diseased pepper plants.

(Concentrations of culture filtrates 0 = 0 %., 1=10%, 2 = 25%, 3=50%)

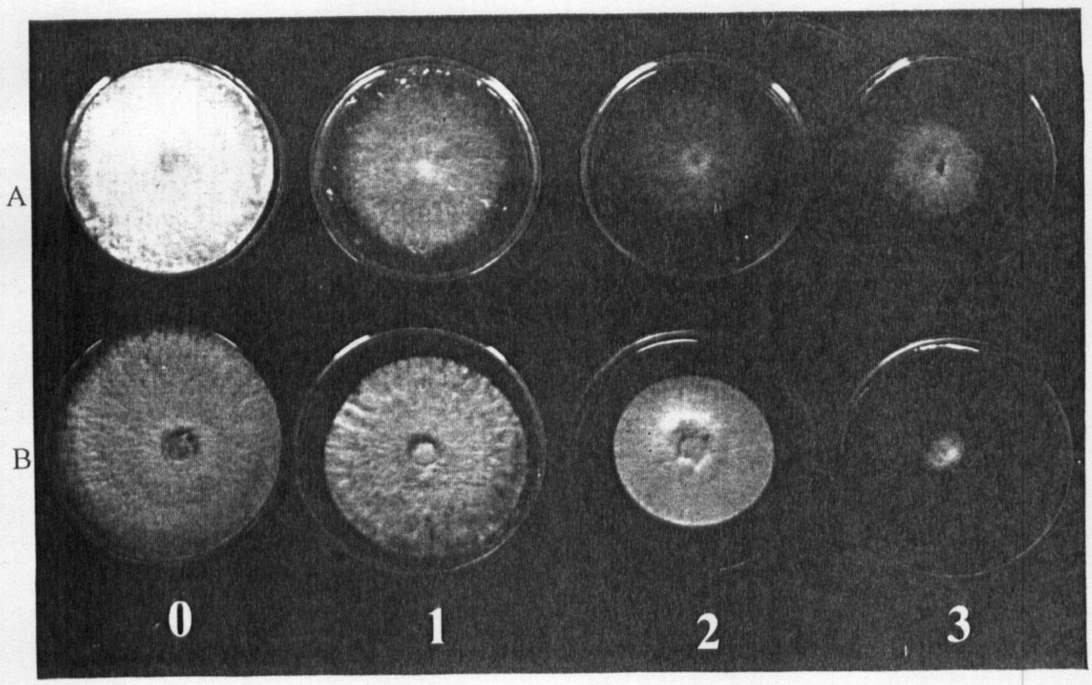


Fig (7): Effect of different culture filtrate concentrations of *T. harzianum* on mycelial growth of *P. aphanidermatum* (A) and *R. solani* (B).

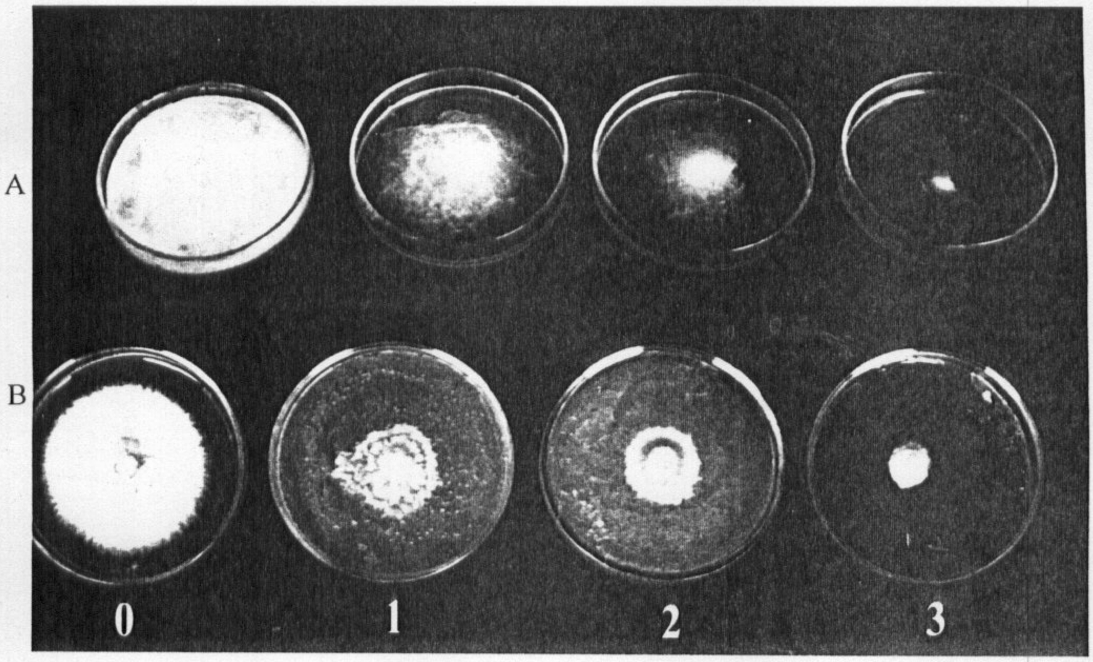


Fig. (8) Effect of different culture filtrate concentrations of *G. virens* on mycelial growth of *P. aphanidermatum* (A) and *R. solani* (B).

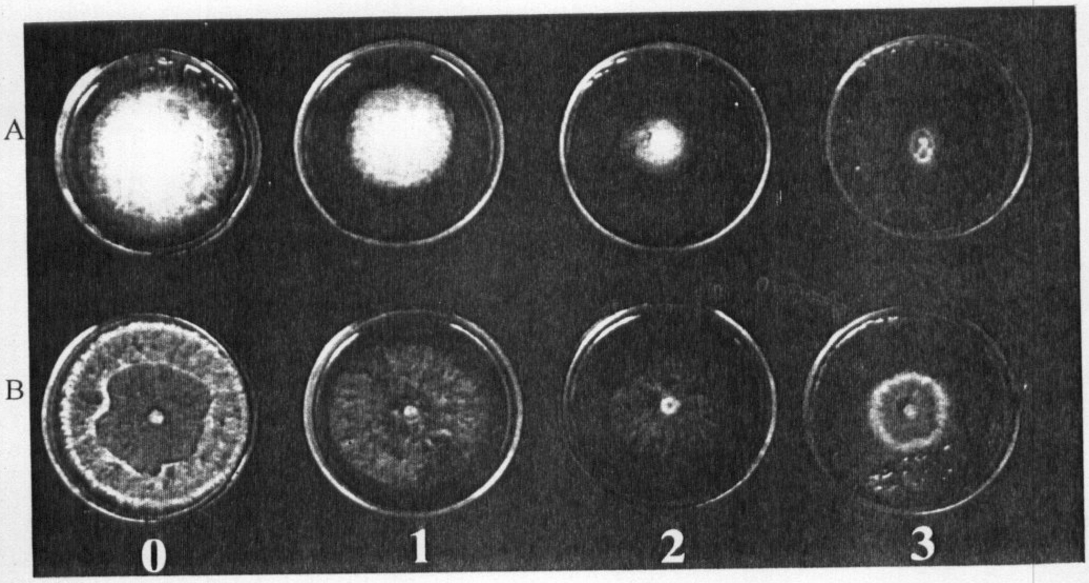
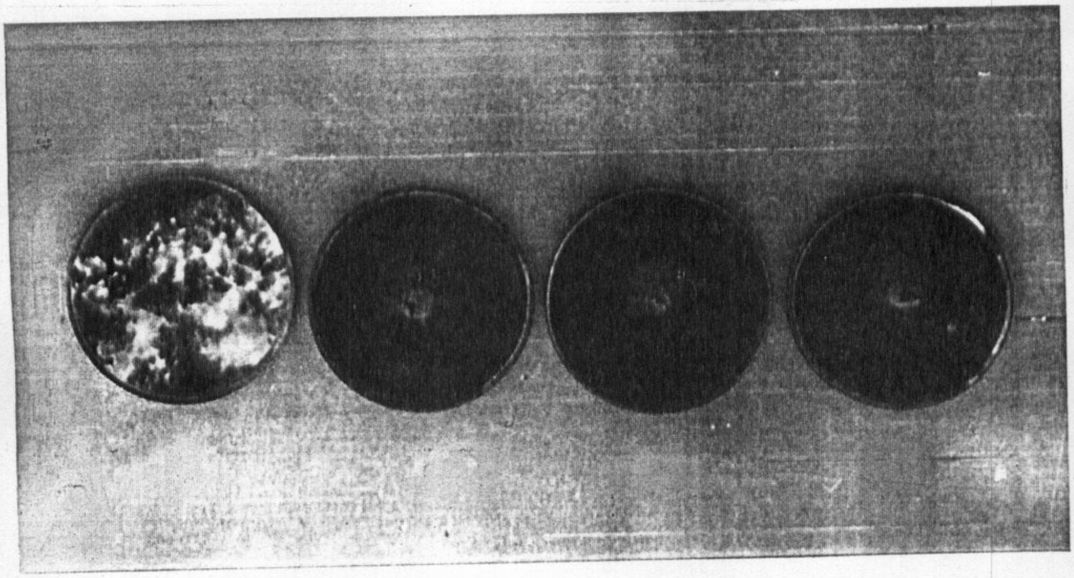


Fig. (9) Effect of different culture filtrate concentrations of *P. fluorescens* on mycelial growth of *P. aphanidermatum* (A) and *R. solani* (B).



0 1 2 3

Fig (10) Effect of different culture filtrate concentrations of an *Actinomycetes sp.* (isolated no. 1) on mycelial growth of *F. solani.*

The more the increase in filtrate concentration, the higher the effect on the pathogenic fungus. (Figs. 5, 6, 7, 8, 9 and 10)

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

Extraction and purification of antifungal compound (s) produced by *G. virens* and *T. harzianum* using thin layer chromatography technique indicated the existence of the antibiotic Gliotoxin by both fungi (Fig. 11). 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).

3.3.1. 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 (10) 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 is also clear from the data that concentration of the antibiotic extracted from *G. virens* was higher than those extracted from *T. harzianum* Fig. (12)

Table (10): Effect of Gliotoxin producted 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		
	Fusarium oxysporum f. sp. *lycopersici*	*Rhizoctonia solani*	*Fusarium solani*	*Rhizoctonia solani*	*Pythium aphanidermatum*
T. harzianum	2.24 b	2.49 ab	1.49 c	2.09 b	2.93 a
G. virens	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.

Table (11): Total phenolic compound in culture filtrates of the different antagonists.

Antagonists	Total phenols/ml
T. harzianum	0.054
G. virens	0.084
P. fluorescens	0.223
B. subtilis	0.068
Actinomycete sp.	0.173

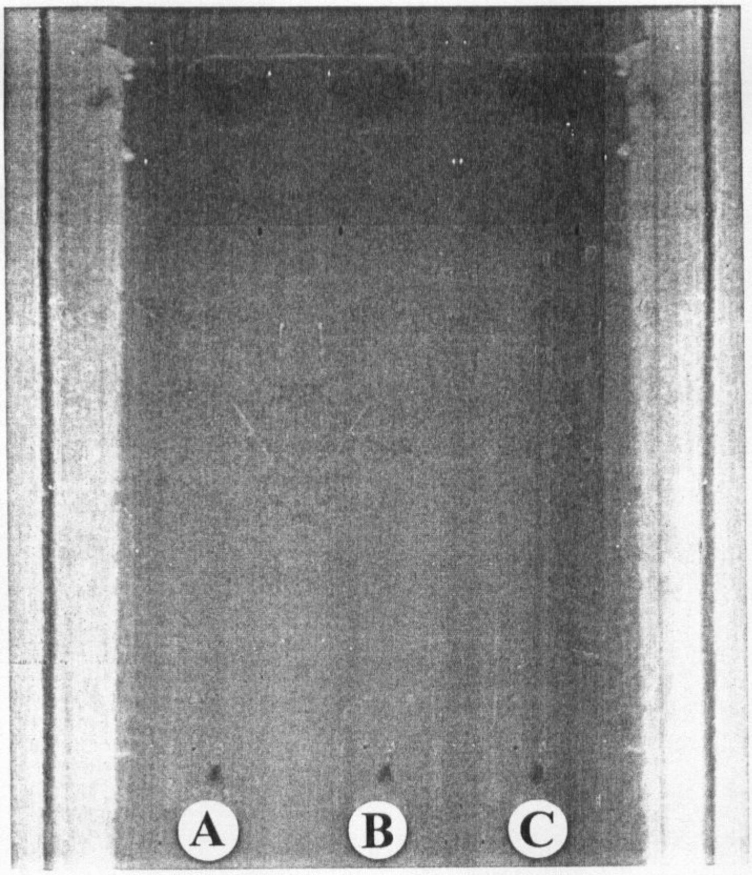


Fig. (11) Detection and extraction of *Gliotoxin* from *G. virens* and *T. harzianum* by thin layer chromatography

A: Gliotoxin (standard)

B: *G. virens*

C: *T. harzianum*

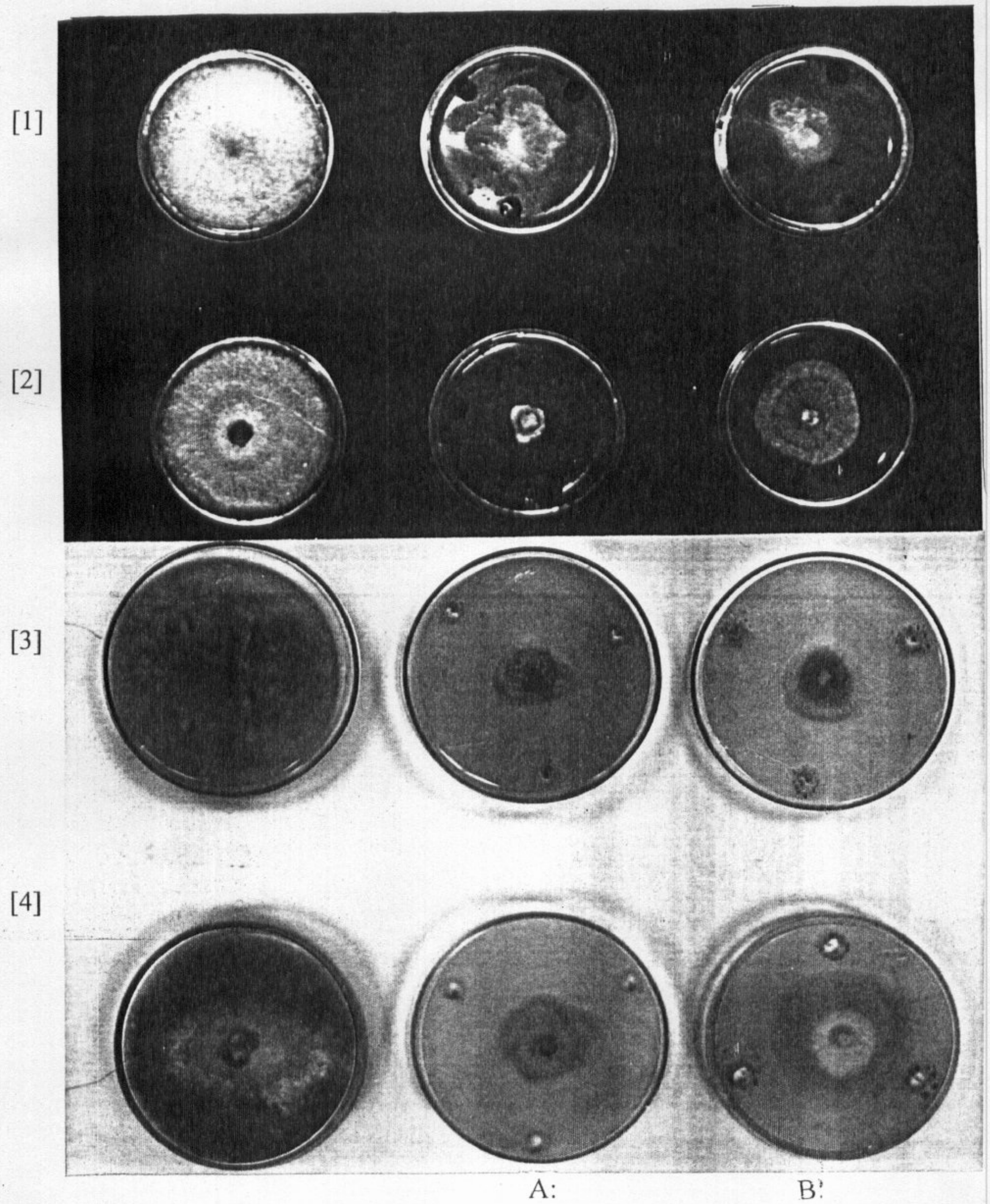


Fig. (12) Effect of Gliotoxin produced by *G. virens* (A) and *T. harzianum* (B) (in holes) on mycelial growth of: *Pythium aphanidermatum* (1), *Rhizoctonia solani* (2), *Fusarium oxysporum* f. sp. *lycopersici*. (3) *Fusarium solani* (4)

3.4. Determination of total phenolic compounds in culture filtrates of different antagonists:

Total phenolic compounds were determined in culture filtrates of the different antagonists using folin-dinnes reagent.

Data presented in Table (11) show that the bacteria *P. fluorescens* proved to have the highest content of phenolic compounds followed by the *Actinomycete* sp. and *G. virens*.

4. The *in vivo* experiments:

4.1. Biological control of soil borne pathogens affecting tomato and pepper plants:

An isolate each of *T. harzianum, G. virens, P. fluorescens, B. subtilis* and *Actinomycetes* sp. were used for controlling damping off and root-rot of tomato and pepper plants under greenhouse conditions during 1997 and 1998 growing seasons.

Data presented in Table (12) indicate that all the tested biocontrol agents as well as the fungicide benomyl significantly reduced post-emergence damping off, root-rot and disease index caused by *F. oxysporum* f. sp. *lycopersici* during 1997 growing season. However benomyle was the most effective in this respect. The same trend was also obtained during 1998 growing season. Whereas no significant differences were observed between the effect of benomyle and *T. harzianum* on the post emergence damping off and disease severity index caused by the pathogen (Fig. 13, 14).

Data presented in Table (13) and Fig. (15, 16) show that *T. harzianum* and *G. virens* were more effective biocontrol agents on controlling the post emergence damping off and disease severity index of

Table (12): Effect of different biocontrol agents on damping off and root-rot diseases of tomato plants caused by *Fusarium oxysporum* f. sp. *lycepersici* 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
Trichoderma harzianum	19.11 ab	31.33 b	21.77 b	16.33 a	33.00 b	19.93 a
Gliocladium virens	20.55 ab	37.44 c	22.33 b	22.15 b	35.66 b	24.63 b
Pseudomonas fluorescens	23.89 b	48.44 d	26.44 c	27.72 c	51.55 c	30.33 c
Bacillus subtilis	23.33 b	51.33 d	26.33 c	30.88 c	55.44 d	33.33 d
Actinomycete sp.	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.

Table (13): 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
Trichoderma harzianum	20.33 b	33.66 a	22.33 ab	19.33 a	39.66 b	22.66 a
Gliocladium virens	21.66 b	39.13 b	29.00 c	24.47 b	40.33 b	26.55 b
Pseudomonas fluorescens	27.33 c	55.66 c	30.33 c	29.33 c	44.55 c	30.44 c
Bacillus subtilis	33.00 d	55.33 c	35.33 d	30.33 c	51.36 d	33.22 c
Actinomycete sp.	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.

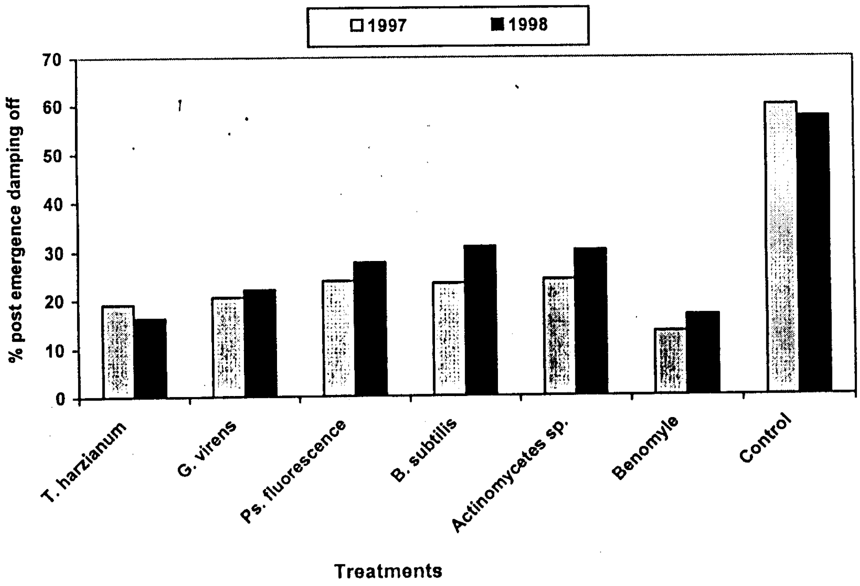


Fig. 13 : Effect of different biocontrol agents on seedling damping off incidence of tomato plants caused by *Fusarium oxysporum* f. sp. *lycopersici* under greenhouse conditions during 1997 and 1998 seasons.

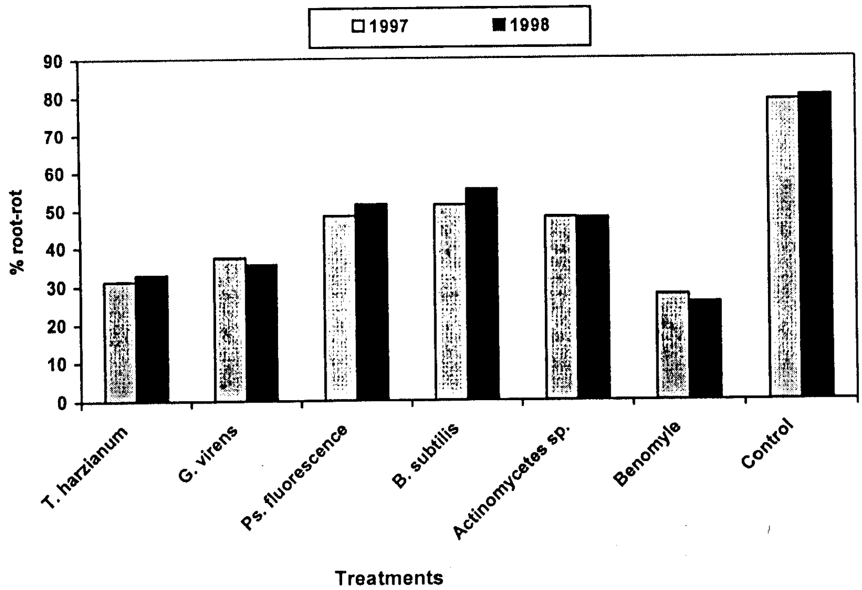


Fig. 14: Effect of different biocontrol agents on root-rot disease incidence of tomato plants caused by *Fusarium oxysporum* f. sp. *lycopersici* under greenhouse conditions during 1997 and 1998 seasons.

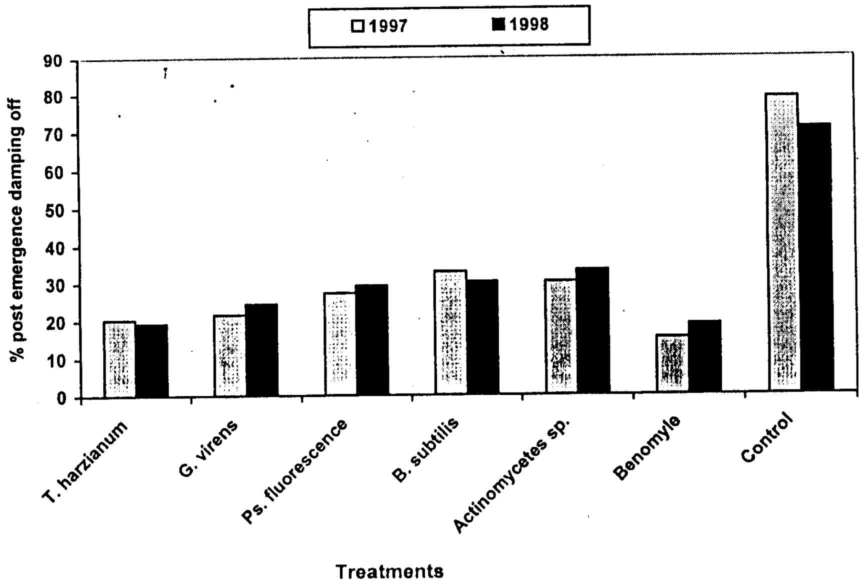


Fig. 15: Effect of different biocontrol agents on seedling damping off incidence of tomato plants caused by *Rhizoctonia solani* under greenhouse conditions during 1997 and 1998 seasons.

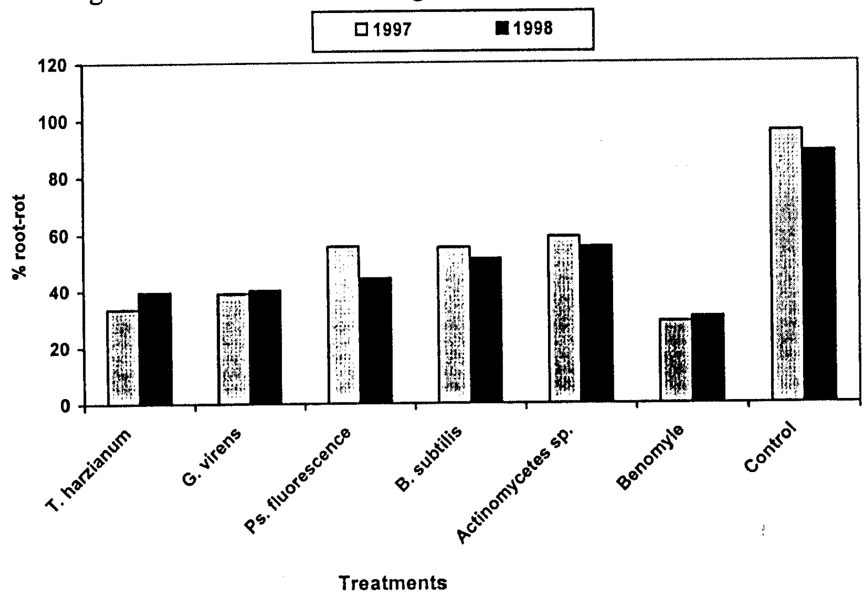


Fig. 16: Effect of different biocontrol agents on root-rot disease incidence of tomato plants caused by *Rhizoctonia solani* under greenhouse conditions during 1997 and 1998 seasons.

tomato seedlings caused by *R. solani* than *P. fluorescens* or *B. subtilis*. However, benomyle gave the best result in this respect during 1997 and 1998 growing seasons.

As far as the effect of the different biocontrol agents on the pathogens of pepper plants is concern, data in Table (14) show that, all the tested biocontrol agents significantly reduced post emergence damping off, root-rot and disease severity index of pepper plants compared with the check treatment (soil infested with *Fusarium solani* only). It is obvious that benomyle was the most effective in this respect followed by *T. harzianum* and *G. virens*, respectively in both seasons (1997 and 1998) Figs. (17, 18).

Data presented in Table (15) and Fig. (19, 20) show that, application of the biocontrol agents in infested soil with *R. solani* significantly reduced post emergence damping off, root-rot and disease index of pepper plants. Among the tested biocontrol agents *T. harzianum* proved to be the best followed by *G. virens*. However, no significant differences was noticed between the effect of benomyle and *T. harzianum* during 1997 and 1998 growing seasons.

Data in Table (16) indicate that application of the tested biocontrol agents to soil infested with *P. aphanidermatum* significantly reduced disease incidence of pepper compared with the non treated pathogen (control). The most beneficial results of biotic agents were obtained by *T. harzianum* and *G. virens*, but in relatively lower degrees compared with the fungicide benomyle during 1997 season. However, the same trend was also obtained during 1998 growing season (Fig. 21, 22).

Table (14): 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
T.richoderma harzianum	15.44 ab	27.00 a	16.33 a	20.92 a	39.33 b	22.00 e
Gliocladium virens	15.98 ab	33.33 b	19.77 ab	23.66 b	40.33 c	26.66 b
Pseudomonas fluorescens	19.89 c	41.44 c	22.22 b	27.55 c	51.44 d	30.33 c
Bacillus subtilis	22.33 c	55.44 d	25.66 b	30.00 d	51.33 d	33.33 c
Actinomycete sp.	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.

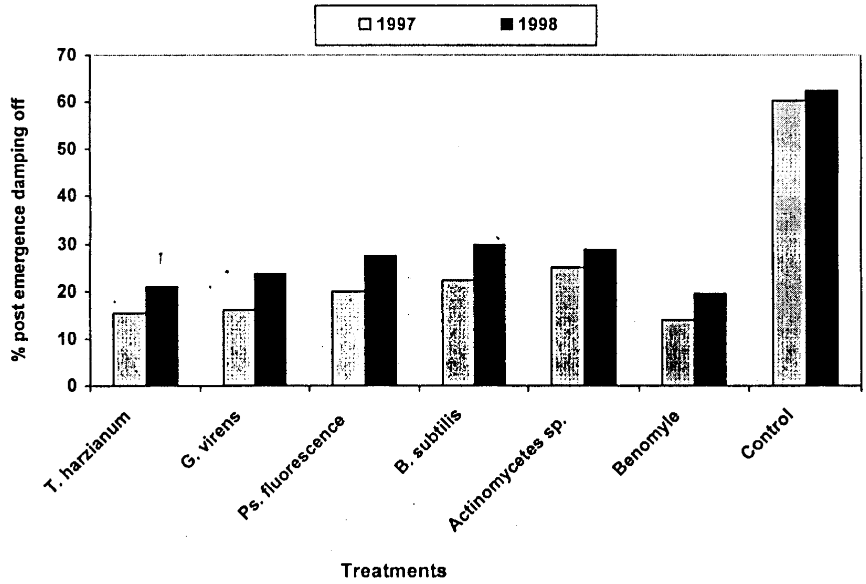


Fig. 17: Effect of different biocontrol agents on seedling damping off incidence of pepper plants caused by *Fusarium solani* under greenhouse conditions during 1997 and 1998 seasons.

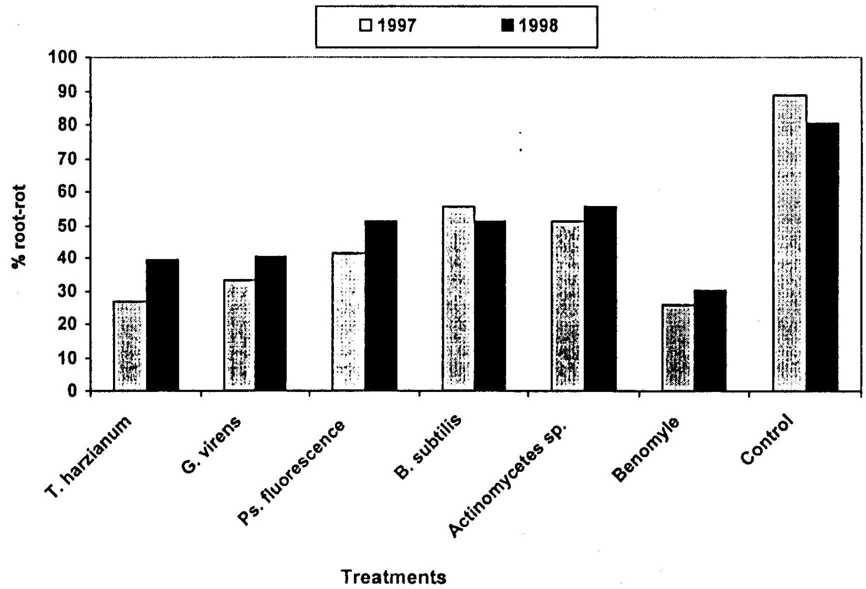


Fig. 18: Effect of different biocontrol agents on root-rot disease incidence of pepper plants caused by *Fusarium solani* under greenhouse conditions during 1997 and 1998 seasons.

Table (15): 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
Trichoderma harzianum	20.66 b	33.66 b	24.33 b	15.33 a	30.33 a	19.55 a
Gliocladium virens	23.33 b	44.44 c	30.33 c	19.66 b	37.33 b	25.33 b
Pseudomonas fluorescens	27.66 c	48.55 d	33.66 c	24.33 c	41.44 c	28.44 b
Bacillus subtilis	30.33 c	55.66 e	33.33 c	26.55	51.55 d	33.33 c
Actinomycete sp.	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.

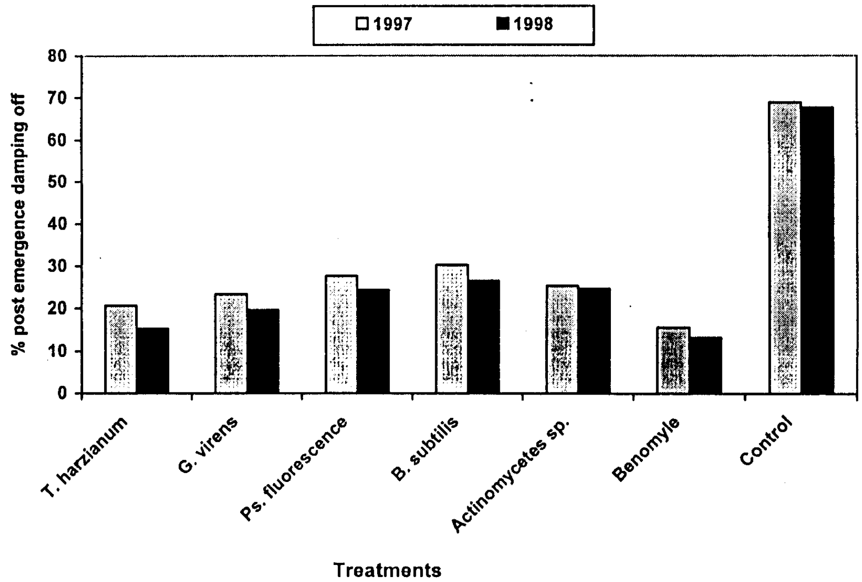


Fig. 19 : Effect of different biocontrol agents on seedling damping off incidence of pepper plants caused by *Rhizoctonia solani* under greenhouse conditions during 1997 and 1998 seasons.

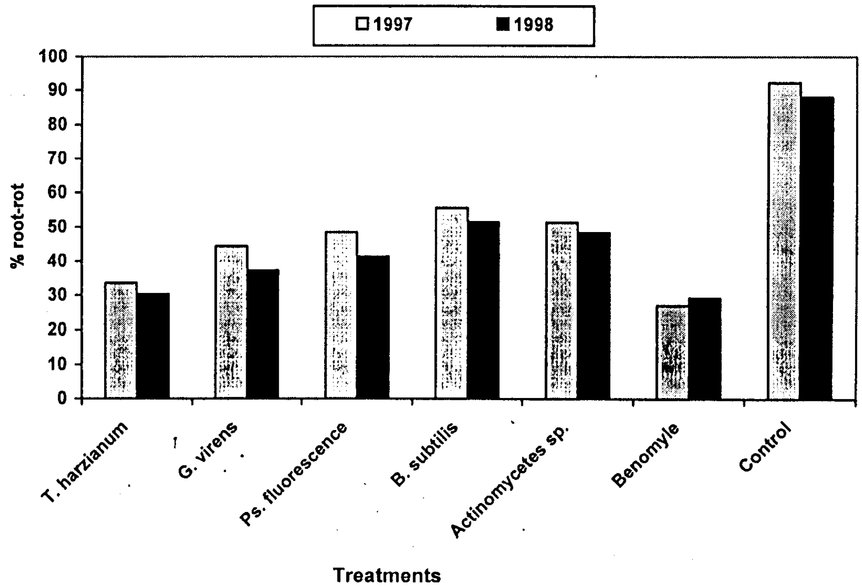


Fig. 20 : Effect of different biocontrol agents on root-rot disease incidence of pepper plants caused by *Rhizoctonia solani* under greenhouse conditions during 1997 and 1998 seasons.

Table (16): 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
Trichoderma harzianum	17.33 a	30.33 a	20.66 a	19.66 b	37.55 b	22.11 a
Gliocladium virens	20.33 a	37.44 b	25.66 b	20.44 b	42.55 c	25.33 b
Pseudomonas fluorescens	26.66 b	45.44 c	30.74 c	26.88 c	55.66 d	33.33 c
Bacillus subtilis	32.33 c	51.55 d	35.11 d	30.66 d	55.88 d	33.33 c
Actinomycete sp.	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.

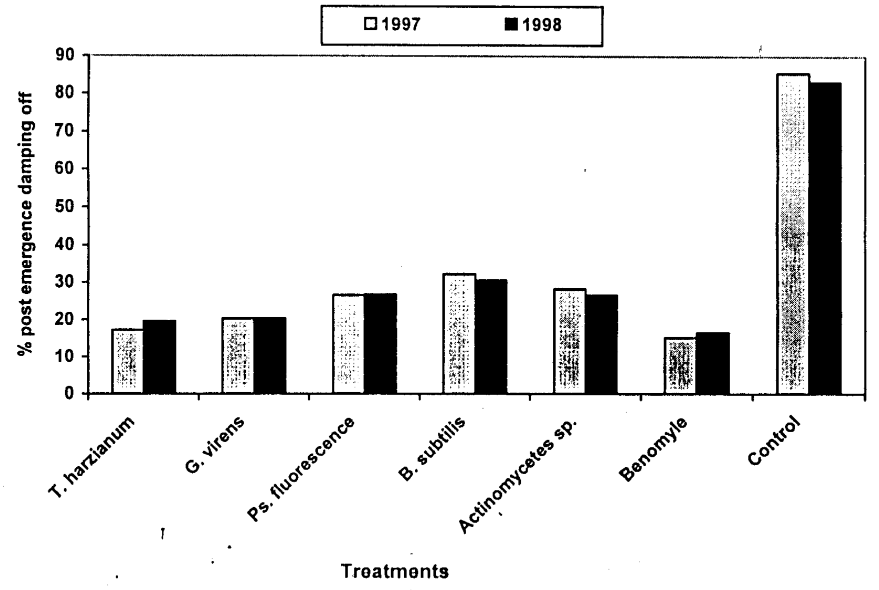


Fig. 21 : Effect of different biocontrol agents on seedling damping off incidence of pepper plants caused by *Pythium aphanidernatum* under greenhouse conditions during 1997 and 1998 seasons.

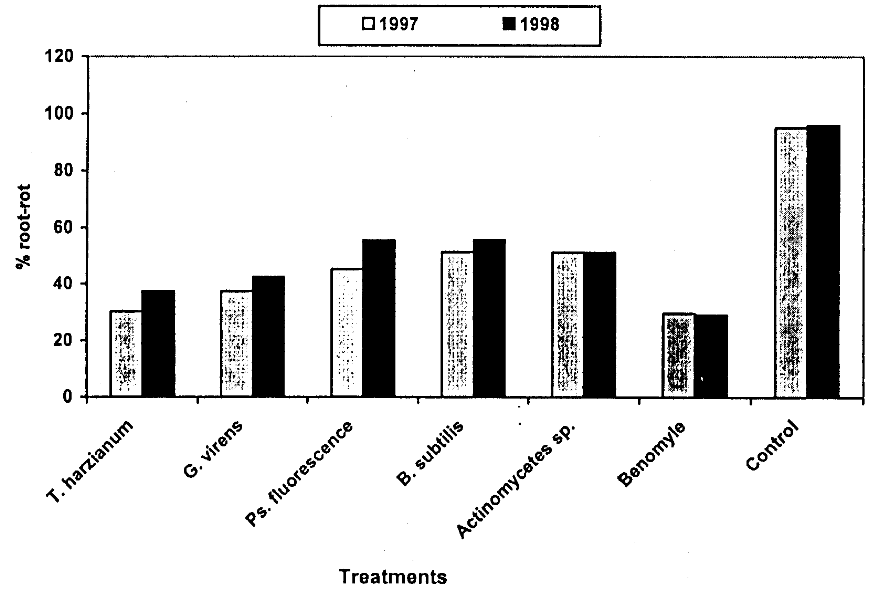


Fig. 22 : Effect of different biocontrol agents on root-rot disease incidence of pepper plants caused by *Pythium aphanidernatum* under greenhouse conditions during 1997 and 1998 seasons.

4.2. Effect of the tested biocontrol agents on morphological characteristics of tomato and pepper plants:

The effect of the tested biocontrol agents on certain morphological characteristics i.e. plant height, average number of leaves, dry weight of shoot and dry weight of root/plant was studied.

4.2.1. Effect of tested biocontrol agents on growth of tomato plants infested with *F. oxysporum* f. sp. *lycopersici*:

Data in Table (17) indicate that application with each of *T. harzianum*, *G. virens*, *P. fluorescens*, *B. subtilis* and *Actinomycete* sp. significantly increased all morphological characteristics of tomato plants in both seasons (1997 & 1998). The best results were obtained with *T. harzianum* and *G. virens* treatments. No significant differences were observed between the effect of benomyle and *T. harzianum* or *G. virens*.

4.2.2. Effect of the tested biocontrol agents on growth of tomato and pepper plants infested with *Rhizoctonia solani*.

1. Tomato plants:

Data presented in Table (18) show morphological characteristics of tomato plants previously infested with *R. solani* and treated with each of different biocontrol agents during 1997 and 1998 seasons. Data show that all treatments significantly increased all morphological characteristics of tomato plants in both seasons. However, treatment with *T. harzianum* was the best among all the tested biocontrol agents.

2. Pepper plants:

Data presented in Table (19) show that significant improvement in plant growth in all treated plants with the antagonists or benomyle.

Table (17): Effect of the different biocontrol agents on morphological characteristics of tomato plants infested with *Fusarium oxysporum* f. sp. *lycopersici* under greenhouse conditions during 1997 and 1998 seasons.

Soil treatments	Season 1997				Season 1998			
	Plant height (cm)	Average no. of leaves/plant	D.W. of shoot (gm/plant)	D.W. of root (gm/plant)	Plant height (cm)	Average no. of leaves/plant	D.W. of shoot (gm/plant)	D.W. of root (gm/plant)
F. oxy. F. sp. lycopersici + *T. harzianum*	42.00 a	35.44 a	16.13 a	5.69 a	41.55 a	38.44 a	18.91 a	5.18 a
F. oxy. F. sp. lycopersici + *G. virens*	40.88 a	34.11 a	16.13 a	4.73 b	40.33 ab	37.11 a	18.40 ab	4.90 a
F. oxy. F. sp. lycopersici + *P. fluorescens*	33.22 b	29.55 b	13.51 b	5.99 a	35.63 cd	34.07 b	14.77 c	5.16 a
F. oxy. F. sp. lycopersici + *B. subtilis*	32.83 b	29.55 b	13.59 b	4.92 abc	34.14 cd	32.55 bc	13.77 cd	3.79 b
F. oxy. F. sp. lycopersici + *Actinomycete sp.*	31.33 b	30.03 b	12.81 b	4.02 bc	33.51 cd	33.33 bc	13.81 cd	3.99 b
F. oxy. F. sp. lycopersici + Benomyle	43.77 a	36.44 a	17.15 a	5.31 ab	44.11 a	38.88 a	17.39 b	5.02 a
F. oxy. F. sp. lycopersici	30.07 c	25.33 c	7.69 c	2.07 d	31.77 d	30.33 c	9.70 e	2.00 c
Uninfested soil	30.55 c	26.77 c	11.19 b	3.88 c	31.92 d	30.44 c	12.90 d	3.08 bc

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

Table (18): Effect of different biocontrol agents on morphological characteristics of tomato plants infested with *Rhizoctonia solani* under greenhouse conditions during 1997 and 1998 seasons.

Soil treatments	Season 1997				Season 1998			
	Plant height (cm)	Average no. of leaves/plant	D.W. of shoot (gm/plant)	D.W. of root (gm/plant)	Plant height (cm)	Average no. of leaves/plant	D.W. of shoot (gm/plant)	D.W. of root (gm/plant)
Rhizoctonia solani + *T. harzianum*	29.50 ab	18.77 a	6.20 a	1.28 abc	31.92 a	19.43 a	6.58 a	1.95 a
Rhizoctonia solani + *G. virens*	27.55 bc	18.11 ab	5.79 ab	1.40 a	28.79 b	18.60 a	6.06 a	1.94 ab
Rhizoctonia solani + *P. fluorescens*	26.00 cd	18.74 a	4.82 bc	1.09 c	26.89 c	17.13 b	4.73 b	1.12 bc
Rhizoctonia solani + *B. subtilis*	24.71 d	16.10 b	4.50 c	1.10 c	22.69 d	16.60 bc	4.66 b	1.04 c
Rhizoctonia solani + *Actinomycets sp.*	26.99 cd	17.05 b	4.73 bc	1.14 bc	247.33 d	16.90 bc	4.33 b	1.09 c
Rhizoctonia solani + Benomyle	30.77 a	19.77 a	4.86 bc	1.34 ab	32.11 a	18.88 a	5.81 a	1.64 a
Rhizoctonia solani	12.16 f	10.88 c	2.425 d	0.40 d	13.55 e	11.98 d	2.83 c	0.56 d
Uninfested soil (control)	22.49 e	14.95 bc	3.83 c	0.98 cd	23.39 d	13.52 c	3.56 c	0.99 c

In the same column, means followed by the same letter are not significantly different at 5% level according to DMRT.

Table (19): Effect of different biocontrol agents on morphological characteristics of pepper plants infested with *Rhizoctonia solani* under greenhouse conditions during 1997 and 1998 seasons.

Soil treatments	1997 season.				1998 season			
	Plant height (cm)	Average no. of leaves/plant	D.W. of- shoot (gm/plant)	D.W. of root (gm/plant)	Plant height (cm)	Average no. of leaves/plant	D.W. of shoot (gm/plant)	D.W. of root (gm/plant)
Rhizoctonia solani + *T. harzianum*	23.33 a	22.33 a	4.97 a	1.25 ab	22.66 a	4.88 bc	4.88 bc	1.13 a
Rhizoctonia solani + *G. virens*	20.05 bc	20.10 ab	5.39 a	1.23 ab	19.52 bc	5.82 a	5.82 a	1.23 ab
Rhizoctonia solani + *P. fluorescens*	17.68 c	21.55 a	3.82 a	1.87 ab	20.59 ab	4.52 cd	4.52 cd	1.11 b
Rhizoctonia solani + *B. subtilis*	14.41 c	16.99 c	4.95 a	1.07 b	18.03 c	4.40 cd	4.40 cd	1.10 b
Rhizoctonia solani + *Actinomycets sp.*	16.82 c	18.16 bc	4.84 a	1.07 b	18.73 c	4.14 cd	4.14 cd	1.15 b
Rhizoctonia solani + Benomyle	22.20 ab	21.49 a	5.59 a	1.29 a	22.00 a	5.57 ab	5.57 ab	1.35 a
Rhizoctonia solani	13.33 d	9.62 d	2.02 b	0.57 c	10.84 d	2.14 e	2.14 e	0.79 bc
Uninfested soil (control)	15.64 c	14.63 c	3.35 ab	0.99 bc	17.66 c	14.66 cd	3.61 d	1.00 bc

In the same column, means followed by the same letter are not significantly different at 5% level according to DMRT.

However *T. harzianum* and *G. virens* were the most effective in this respect followed by *P. fluorescens in both seasons.*

4.2.3. Effect of the tested biocontrol agents on growth of pepper plants infested with *Fusarium solani*:

Data presented in Table (20) show that all treatments with the fungicide benomyle or each of the tested biocontrol agents significantly improved the studied morphological characteristics. No significant differences between the effect of *T. harzianum, G. virens* and benomyle in this respect during the both seasons (Fig. 23).

4.2.4. Effect on growth of pepper plants infested with *Pythium aphanidermatum*:

Data in Table (21) indicate that application of different biocontrol agents in soil infested with *P. aphanidermatum* significantly improved the growth of pepper plants. However, *T. harzianum* and *G. virens* were the most effective in this respect followed by *P. fluorescens* and *B. subtilis*, respectively. No significant differences were found between the effect of benomyle, *T. harzianum* and *G. virens* on enhancing the growth of pepper plants.

In season 1998, all treatments significantly improved the growth of pepper plants compared with the check treatment. *T. harzianum* was the most effective treatment in this respect (more effective than benomyle) (Fig. 24).

Table (20): Effect of the different biocontrol agents on morphological characteristics of pepper plants infested with *Fusarium solani* under greenhouse conditions during 1997 and 1998 seasons.

Soil treatments	Season 1997				Season 1998			
	Plant height (cm)	Average no. of leaves/plant	D.W. of shoot (gm/plant)	D.W. of root (gm/plant)	Plant height (cm)	Average no. of leaves/plant	D.W. of shoot (gm/plant)	D.W. of root (gm/plant)
Fusarium solani + *T. harzianum*	33.77 a	34.44 a	13.11 a	4.19 a	33.14 a	32.03 a	12.40 a	4.50 a
Fusarium solani + *G. virens*	33.11 a	33.22 a	10.80 b	4.16 a	31.44 a	32.56 a	12.55 a	4.44 ab
Fusarium solani + *P. fluorescens*	25.77 b	31.00 b	10.79 b	3.66 a	26.44 b	25.74 b	10.66 b	3.80 ab
Fusarium solani + *B. subtilis*	25.44 b	26.33 c	10.28 b	3.06 b	25.23 bc	26.49 b	10.13 b	3.53 bc
Fusarium solani + *Actinomycete sp.*	25.66 b	28.99 bc	10.02 b	3.03 b	25.00 bc	27.00 b	10.03 b	3.33 bc
Fusarium solani + Benomyle	33.10 a	34.72 a	13.04 a	4.03 a	33.19 a	33.00 a	12.93 a	4.73 a
Fusarium solani	22.66 c	20.88 d	6.87 d	2.14 c	22.81 d	18.51 d	6.11 c	2.43 d
Uninfested soil	24.66 b	23.11 c	9.07 c	2.99 bc	24.19 cd	22.60 c	9.71 b	2.68 cd

In the same column, means followed by a common letter in the same column are not significantly different at the 5% level acording to DMRT.

Table (21): Effect of the different biocontrol agents on morphological characteristics of pepper plants infested with *Pythium aphanidermatum* under greenhouse conditions during 1997 and 1998 seasons.

Soil treatments	Season 1997				Season 1998			
	Plant height (cm)	Average no. of leaves/plant	D.W. of shoot (gm/plant)	D.W. of root (gm/plant)	Plant height (cm)	Average no. of leaves/plant	D.W. of shoot (gm/plant)	D.W. of root (gm/plant)
Pythium aphanidermatum + *T. harzianum*	19.89 a	22.88 a	5.04 a	1.24 a	20.97 a	23.33 a	5.15 a	1.71 a
Pythium aphanidermatum + *G. virens*	20.11 a	21.27 ab	4.64 ab	1.29 a	21.11 a	20.89 b	50.35 a	1.45 ab
Pythium aphanidermatum + *P. fluorescens*	17.55 ab	19.77 bc	4.73 ab	1.13 a	18.40 b	19.11 c	4.77 ab	1.15 bc
Pythium aphanidermatum + *B. subtilis*	17.31 ab	19.21 bc	449 ab	1.8 a	18.10 b	17.21 d	4.32 b	1.11 bc
Pythium aphanidermatum + *Actinomycete sp.*	16.21 b	19.05 bc	3.77 b	1.02 a	17.22 b	17.55 d	4.11 b	1.21 bc
Pythium aphanidermatum + *Benomyle*	19.88 a	23.44 a	4.77 ab	1.27 a	21.31 a	21.55 b	4.93 ab	1.46 ab
Pythium aphanidermatum	14.16 c	11.55 d	2.72 c	0.58 b	15.59 c	13.00 e	2.93 c	0.81 c
Uninfested soil	15.29 bc	17.21 c	3.35 bc	1.01 a	16.41 bc	16.91 d	3.25 c	1.01 bc

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

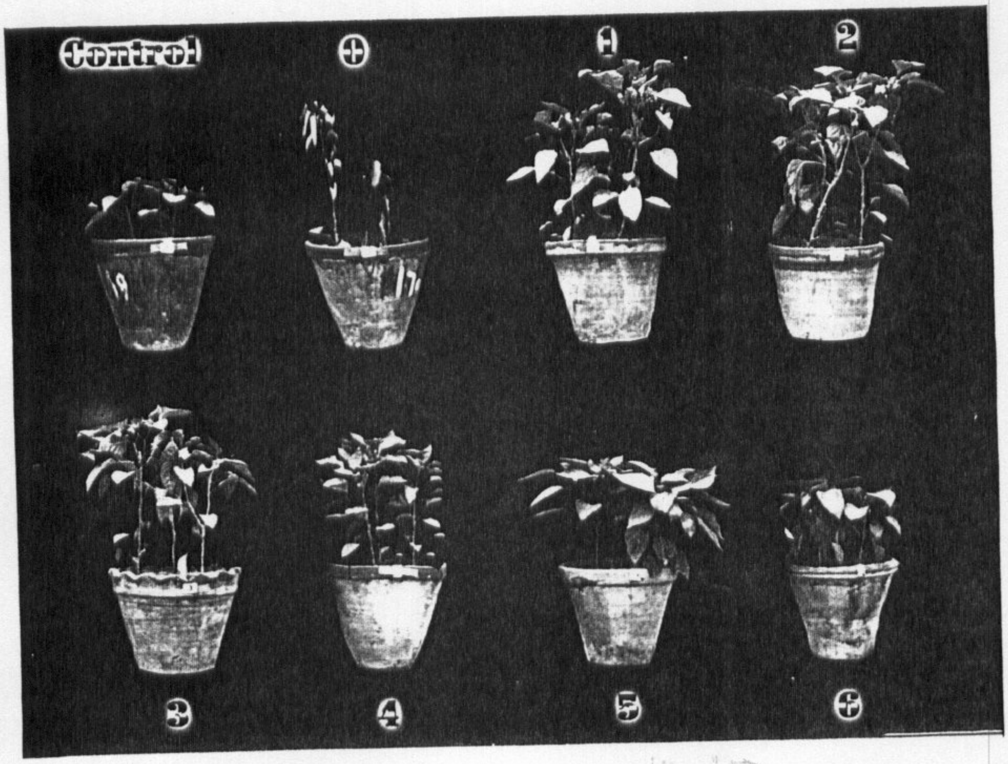


Fig. (23) Effect of different biocontrol agents on growth of pepper plants infected with *F. solani*.

Control: Untreated plants.
0 = Infected with the pathogen only
1 = Benomyle treatment
2 = *T. harzianum* (T_1)
3 = *G. virens* (G_1)
4 = *P. fluorescens* (P_{35})
5 = *B. subtilis* (B_5)
6 = *Actinomycete* sp. isolated no. (1)

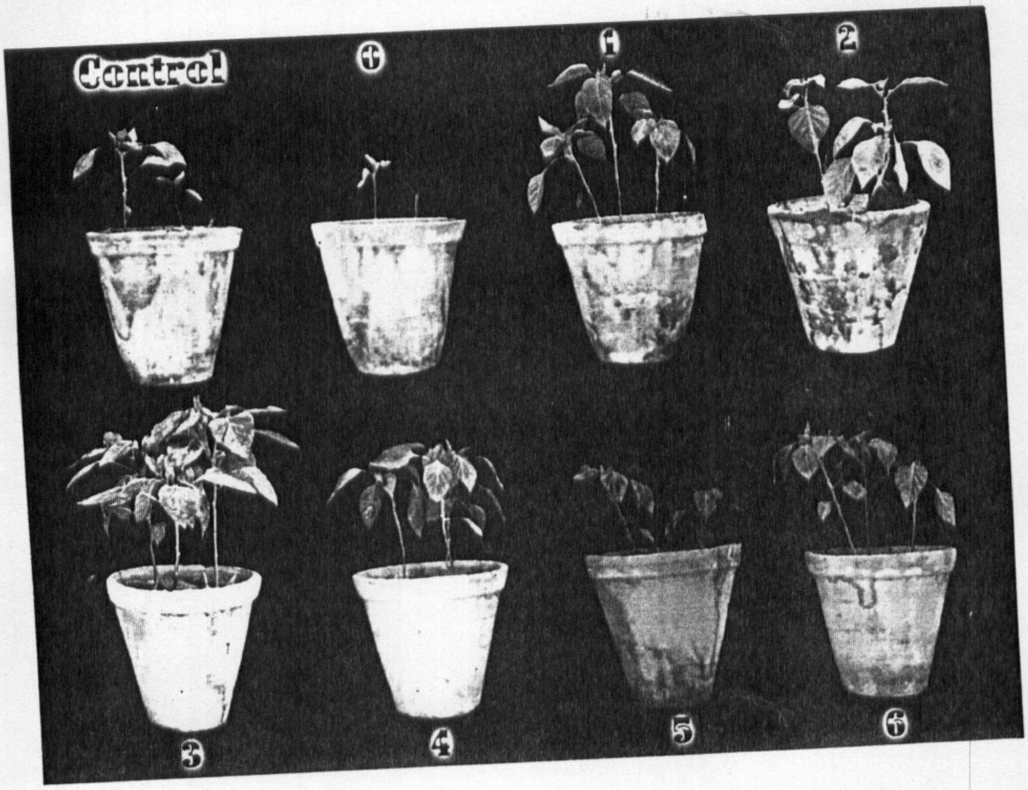


Fig. (24) Effect of different biocontrol agents on growth of pepper plants infected with *P. aphanidermatum*.

Control: Untreated plants.
0 = Infected with the pathogen only
1 = Benomyle treatment
2 = *T. harzianum* (T_1)
3 = *G. virens* (G_1)
4 = *P. fluorescens* (P_{35})
5 = *B. subtilis* (B_5)
6 = *Actinomycete* sp. isolated no. (1)

DISCUSSION

Tomato (*Lycopersicon esculentum*) and pepper (*Capsicum annum*) plants are subject to the attack by many soil borne pathogens. Results of the present work based on several isolates of various fungi associated with tomato and pepper rotted roots which were collected from four Governorates, namely Kafr El-Sheikh, El-Gharbiya, El-Dakahliya and El-Behira. The pathological behavior of the isolated fungi, as well as their characteristics were in agreement with those known for *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., *Alternaria* spp., *Sclerotium* spp. and *Verticillium* spp. However, *Rhizoctonia solani, Fusarium spp* and *Pythium* spp. were the most frequently isolated pathogens.

These results coincide with the findings of **Alavi *et al.*, (1986); Kapoor (1987); Jarvis (1989); Khalifa (1991) and Abada (1994).**

The obtained results revealed that the occurrence and frequency of the isolated fungi differed from on location to another. This may be due to the prevailing environmental conditions during the growing season of tomato and pepper in addition to the crop rotation and the previous cultivated crops.

The *in vivo* studies showed that *F. oxysporum* f. sp. *lycopersici* and *R. solani* were the major soil borne pathogenic fungi of tomato, whereas *Fusarium solani, Rhizoctonia solani* and *Pythium aphanidermatum* were the major soil borne pathogens of pepper plants. *F. oxysporum* f. sp. *lycopersici* was found to attack the plants at any stage of growth causing great economic losses. Similar findings about the destructive action of such fungus on tomato were reported by **Jenkins and Averre (1983); Brammall and Higgins (1985); Jarvis (1989); Kapoor and Kar (1989) and Khalifa (1991).**

Rhizoctonia solani was found the most pathogenic fungus which caused severe damage to tomato and pepper plants during the early weeks after planting. This finding is in agreement with **Alavi *et al.* (1986); Favrin *et al.* (1988); Blancard *et al.* (1991) and Hadwan and Khara (1992).**

Fusarium solani was found to cause pre and most emergence damping off as well as root-rotting of pepper plants. Similar results were obtained by **Koleva and Vitanove (1990); Abada (1994) and Kheirella (Zienab) *et al.* (1994).**

Pythium aphanidermatum was found to be highly pathogenic to pepper plants causing pre-emergence damping off. This finding agreed with reports of **Ibrahimallari (1987); Favrin *et al.* (1988) and Abada (1994).**

Application of chemical fungicides to protect plants against soil-borne pathogenic fungi was and still the primary means to control soil-borne diseases. However, the use of fungicides is becoming more controversial for pollution of soil and consequently become health hazardous. Therefore the search for non-chemical methods for protection against soil borne pathogens was the main target of the present study.

Therefore, several samples from the soil rhizosphere of healthy tomato and pepper plants grown in different locations were collected and screened for the existence of antagonists against the soil-borne pathogenic fungi isolated from tomato and pepper plants.

The obtained results proved resulted in the isolation of 45 bacterial and 12 fungal isolates exhibiting marked antifungal activity against the tested pathogens. The predominate antifungal bacterial isolates were belonging to *Bacillus* spp., *Pseudomonas* spp. and *Actinomycetes* spp. The fungal isolates were belonging to *Trichoderma* spp., *Gliocladium* spp., *Paecilomyces* sp., *Myroithecium* spp. and *Geotrichum* sp.

Results indicated that bacterial isolates *Busillus subtilis* ($B_{.5}$), *Pseudomonas fluorescens* ($P_{.35}$) and an *Actinomycete* sp. (Act. 1) as well as fungal isolates *Trichoderma harzianum* (T_1) and *Gliocladium virens* (G_1) were the most effective biocontrol agents against the tested phytopathogenic fungi. The maximum inhibition zone was recorded with *Actinomycete* sp. against the tested pathogenic fungi, followed by *P. fluorescens* as well as *G. virens* as fungal antagonist. Such antagonistic activity of *Trichoderma* spp. and *Gliocladium* spp. could be related to their ability to act as mycoparasite, produce antibiotics and have an enzyme system capable of attacking a wide range of plant pathogens. This finding was also observed by **Bell *et al.* (1982)** who reported that *Trichoderma* spp. had a high effect against *R. solani* when grown over the pathogen or induce some of inhibition zone. *Trichoderma* spp. was also reported to suppress the germination of *R. solani* sclerotia **(Papavizas, 1985)** and chlamydospores of *Fusarium* spp. **(Comporota, 1985)**. **Chambers and Scott (1995)** found that *Trichoderma* spp. and *G. virens* were mycoparasitism and over grew on *F. oxysporum* f. sp. *lycopersici*.

On the other hand, antibiosis is potentially a principal component of the mechanism of *Trichoderma* spp. and *Gliochaldium* spp.

Pseudomonas spp. inhibited different pathogens by producing antibiotics and/or siderophore which were suppressive to a large number of phytopathogenic fungi **(Hasegawa *et al.* 1990 and Laha *et al.*, 1992)**.

These metabolites includes hemipyocianine, chlororaphin, phenazine 1-carboxylic acid, pyrrolnitrin, pyoluteavin, cyanide and pseudane **(Howell and Stipanovic, 1980; Kloepper and Schorth, 1981; Farvel, 1988 and Kraus and Loper, 1992)**.

Bacillus spp. by their abilities to produce spores tolerating severe condition were recommended as biocontrol agents in general and *B. subtilis* in particular appears to be the most effective as a biocontrol agent **(Broadbent and Baker, 1969)**. Also, *Bacillus* spp. produce many antibiotic which suppress many bacteria and fungi (**Loeffer *et al.*, 1986; Kapoor and Kar, 1989 and Ferreira *et al.*, 1991**). On the other hand, **Mansour (1997)** found that *B. subtilis* had a moderate effect against *F. solani*.

Presence and role of *Actinomycetes* spp. in the rhizosphere have been widely studied and their role as biocontrol agents of soil borne fungal disease was mentioned by **Saracchi *et al.* (1992) and Dormann, (1993)**.

In the present study adding filtrates of antagonistic bioagents at different concentrations to the media led to reduction of mycelial growth of the tested pathogenic fungi. The suppression was more pronounced with the culture filtrates of *P. fluorescens*, *Actinomycete* sp. and *T. harzianum*. It was found that the more the effect, the higher the concentration of the culture filtrate to be tested. However, culture filtrate of *B. subtilis* had the least effect in inhibiting fungal growth of the tested 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. The results are in a harmony with **Khara and Hadwan (1990); Roberts and Lumsden (1990); Bhardwaj *et al.* (1992); Khalifa, (1997) and Hagage, Wafaa, (1998)**.

The implication of antibiosis in biocontrol mechanism has been clearly demonstrated. Data of the present work revealed that either *G. virens* or *T. harzianum* produced the antibiotic gliotoxin in their culture filterates. *Trichoderma* spp. produced many antifungal and antibacterial compounds, i.e. viridin, Trichodermin, suskacillin, dermadin (**Elad *et al.*, 1982, Sivan and Chet, 1987 and Bhardwaj *et al.*, 1992**). Also, *G. virens* produces an array of metabolites including gliotoxin, gliovirin, gliocladic acid, viridin and viridial **(Howell, 1982; Howell and Stipanovic, 1983 and Howell and Stipanovic, 1995)**.

Gliotoxin specifically has been implicated in biocontrol mechanisms **(Abd El-Moity, 1981; Lifshitz *et al.*, 1986; Smith *et al.*, 1990; Howell, 1991 and Lumsden *et al.*, 1992)**.

Gliotoxin is likely to be responsible for biocontrol activity *in vitro* against soil-borne pathogenic fungi. This result is in agreement with **Howell (1991); Lumsden *et al.* (1992) and Howell and Stipanovic (1995)**.

The ability of antagonistic microorganisms to inhibit pathogens through production of wide variety of secondary metabolites may be important in disease suppression. The prominent metabolites consistently associated with antagonistic microorganisms were identified by mass spectrophotometer as a mixture of phenols. These compounds had biological activity against pathogens. **Excoffier *et al.* (1991)** observed that low molecular weight of phenols produced by *Trichoderma* spp. were responsible for inhibiting hydrolytic enzymes of pathogens. **Lumsden *et al.* (1992)** found that phenolic compounds were also produced by *G. virens*. The present results revealed that *P. fluorescens* had the highest content of phenolic compounds followed by *Actinomycete* sp. and *G. virens*.

The used antagonists, *T. harzianum* (T_1), *G. virens* (G_1), *P. fluorescens* ($P._{35}$), *B. subtilis* ($B._5$) and *Actinomycete* sp. (Act. I) which proved *in vitro* to be the most effective against the tested pathogenic fungi were tested *in vivo* to evaluate their effect in controlling diseases incited by those pathogens. Results of pot experiments conduced for this purpose confirmed the efficiency of the tested antagonists. These antagonists had significant effect in protecting tomato and pepper plants against each of the studied pathogens. Level of protection achieved was comparable with those obtained by the application of the recommended dose of the fungicide benomyle. However, *T. harzianum* and *G. virens* were more effective than other tested antagonists. *B. subtilis* had the least effect in this respect. This finding may lead to the use of biological control of soil-borne pathogens instead of the fungicides in the field to avoid risks of such chemicals. These results are in general agreed with those of **Kim and Roh (1987); Sivan and Chet (1987); Khalifa (1991); Phae *et al.* (1992); Gamliel and Katan (1993), Benhamou *et al.* (1996) and Khalifa and Liddell, (1996).**

The growth response of plant is usually determined by measuring plant length, No. of leaves, dry weight of shoot and dry weight of root system for plant. Results of the present study indicated that the tested antagonists used against *F. oxysporum* f. sp. *lycopersici* and *R. solani* showed significant increases of growth of tomato plants. However, *T. harzianum*, *G. virens* and *P. fluorescens* gave more developed and vigorous plants than the other used antagonists. Also, the results showed the same trend with pepper plants whereas, the same antagonists were used against *F. solani*, *R. solani* and *P. aphanidermatum*. These results could be attributed to the effect of the used antagonists in controlling soil-borne diseases in addition that they might possess growth regulating substance(s) which stimulate growth of such plants.

Becker and Cook, (1988) indicated that the plant growth-promoting activity of some strains of fluorescent Pseudomonads on wheat resulted from ability of the strains to suppress *Pythium ultimum* by production of siderophores, including in relatively low-pH soil. Also, it was reported that *Trichederma* spp. produced a growth-regulating factor that increases the rate of seed germination and dry weight of shoot and stems of tomato and tobacco plants (**Windham *et al.*, 1986; Shahida-Parveen *et al.* 1991 and Khalifa and Liddell, 1996**).

SUMMARY

Tomato (*Lycopersicon esculentum*) and Pepper (*Capsicum annum*)plants are vulnerable to several soil-born pathogens causing damping-off and root-rot diseases. These pathogens cause damage to plants and hence to subsequent reduction in fruit yield. Seed treatment fungicides are commonly used for controlling such diseases. Because of the hazardous effects of chemicals to the human and the environment. Therefore, new approaches were necessary for controlling these pathogens which attack tomato and Pepper plants. Biological control of such disease by certain biocontrol agents was the most essential approach in this respect.

The obtained results of the present work are summarized as follows:

1. Isolation trails from naturally rotted roots and crowns of tomato and pepper plants collected from different Governorates in the Delta of Egypt, i.e., Kafr El-Sheikh, Gharbiya, Dakahliya and Behira yielded 60 fungal isolates belong to 6 genera namely, *Fusarium spp., Rhizoctonia spp., Pythium spp., Alternaria spp., Sclerotium spp.* and *Verticillium spp.*

2. Occurrence and frequency of fungi associated with diseased samples differed according to the locality from which the samples were collected. The highest number of fungi was isolated from samples collected from Kafr El-Sheikh Governorate (25 isolates). The most frequent fungi were *Fusarium spp., Rhizoctonia spp.* and *Pythium spp.*

3. The pathogenicity tests and identification of the isolated pathogens revealed that *F. oxysporum* f. sp. *lycopersici* and *R. solani* are the major soil-borne pathogenic fungi of tomato, whereas these of pepper were *Fusarium solani*, *Rhizoctonia solani* and *Pythium aphanidermatum*.

   These pathogens are implicated in damping off, root-rots and wilting of tomato and pepper plants.

4. The preliminary screening of several 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.

   These biocontrol agents belonged to *Bacillus spp.*, *Pseudomonas spp.* and actinomycetes isolates as bacterial antagonists, whereas fungal isolates were belonging to *Trichoderma spp.*, *Gliocladium spp.*, *Myrothecium spp.*, *Paecilomyces spp.* and *Geotrichum spp.*

5. These antifungal isolates obtained in the preliminary screening were subjected to a standardized test to select those having the highest effect against the tested pathogenic fungi, they were identified as, *Trichoderma harzianum* (T.1), *Gliocladium virens* (G. 1), *Pseudomonas fluorescens* (P. 35), *Bacillus subtilis* (B. 5) and Actinomycete isolate (Act. 1).

6. Sterilized culture filtrates of most antagonists significantly reduced the growth of tested pathogenic fungi when added to media. The antagonistic effect increased with the increase of concentration. The culture filtrates at 50% concentration of *P. fluorescens*, *Actinomycete* isolate and *T. harzianum* had higher inhibitory effects.

7. The antibiotic gliotoxin was found to be produced by *G. virens* and *T. harzianum* as a metabolite produced by both fungi. The antibiotic gliotoxin exerted an inhibitory action against the tested pathogenic fungi.

8. The highest percentage of total phenols found in culture filtrates of *P. fluorescens,* Actinomycet isolate 1 and *G. virens* were 0.223%, 0.173% and 0.084%, respectively.

9. The antifungal isolates *T. harzianum* (T. 1), *G. virens* (G.1), *P. fluorescens* (P. 35), *B. subtilis* (B. 5) and the Actinomycete isolate (1) which proved *in vitro* to be the most effective against the pathogenic fungi, were tested *in vivo.* Results of pot experiments confirmed the efficiency of these antifungal isolates. Significant levels of protection of tomato plants against *F. oxysporum* f. sp. *Lycopersici* and *R. solani* were achieved by the application of the tested selected antagonists. Levels of protection achieved were comparable with those obtained by the recommended dose of the fungicide benomyle.

10. Similar levels of protection of Pepper plants against *F. solani* or *R. solani* or *P. aphanidermatum* could be achieved by the selected tested antagonists. There was no significant differences between protection achieved by the antagonists and the fungicide was obtained.

11. Biocontrol agents were more effective than fungicidal treatment in enhancing growth of tomato and Pepper plants. The different morphological characters i.e. plant length, number of leaves and dry weight of shoot and root per plant were significantly increased due treatment with the tested bioagents. Application of *T. harzianum* was the best treatment followed by *G. virens* and *P. fluorescens*.

Results of the present study provide sufficient evidence to recommend the use of the antifungal isolates *T. harzianum* (T. 1), *G. virens* (G. 1), *P. fluorescens* (P. 35), *B. subtilis* (B. 5) and *Actinomycete sp.* (Act. 1) as successful biocontrol agents against soil-born fungal diseases of tomato and Pepper plants and enhance the growth of both plants.

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ARABIC SUMMARY

الملخص العربى

تتعرض نباتات الطماطم والفلفل لمهاجمة العديد من الفطريات الممرضة القاطنة بالتربة والتى تسبب لها العديد من الامراض وأهمها مرض عفن الجذور والذبول الذى يلحق خسارة كبيرة بمحصول كلا النباتين. والطريقة الشائعة فى مكافحة هذه الأمراض هى استعمال المبيدات إلا أن ضررها الشديد على الإنسان والبيئة حد من استخدامها وأصبح من الضرورة إيجاد بدائل جديدة لمكافحة هذه الأمراض. وتأتى المكافحة الحيوية أولى هذه البدائل حيث أنها تقاوم الأمراض النباتية دون تأثير سلبى على صحة الإنسان أو البيئة. لهذا فقد أجريت هذه الدراسة لمحاولة استخدام بعض الكائنات الحية الدقيقة الموجودة فى التربة للوصول إلى مكافحة حيوية فعالة ضد بعض مسببات الأمراض التى تعيش بالتربة فى المعمل والصوبه.

ويمكن تلخيص النتائج المتحصل عليها كالآتى:

- تم عزل عدة مسببات مرضية من النباتات المصابة التى جمعت من بعض المحافظات فى مصر وهى كفرالشيخ – الغربية – الدقهلية – البحيرة ، كانت محصلتها ٦٠ عزلة فطرية تندرج تحت الأجناس أنواع من فيوزاريوم ، رايزكتونيا ، ببيثيوم ، الترناريا ، فيرتسيليوم ، سكليروشيوم *Fusarium* spp., *Rhizoctonia* spp., *Pythium* spp., *Alternaria* spp., *Verticillium* spp., and *Sclerotium* spp. وتبين من حصر هذه المسببات المرضية أن تكرار المسبب يختلف من موقع إلى آخر وكان أكثر هذه الفطريات انتشارا هى *Fusarium* spp., *Rhizoctonia* spp., and *Pythium* spp.
- ثبت من اختبارات القدرة المرضية وعمليات التعريف أن المسببات المرضية الرئيسية المحمولة بالتربة والتى تصيب نباتات الطماطم هى *Fusarium oxysporum f.* sp. *lycopersici* and *Rhizoctonia solani* أما فى نباتات الفلفل فقد كانت *Fusarium solani, Rhizoctonia solani* and *Pythium aphanidermatum* وتسبب هذه الفطريات مجموعة من الأمراض تشمل موت البادرات وأعفان الجذور والذبول.
- تم عزل العديد من العزلات من عينات التربة التى جمعت من مناطق حول جذور نباتات الطماطم والفلفل السليمة تتمثل فى ٤٥ عزلة بكتيرية تندرج تحت الأجناس. سيدوموناس ، باسيللس ، الاكتينوميسيتات *Pseudomonas* spp., *Bacillus* spp. and *Actinomycetes* spp. على الترتيب ، و ١٢ عزلة فطرية تندرج تحت الأجناس التاليه ترايكودرما ،

جليوكلاديـــــــــوم ، ميروثيســــــيوم ، باسيلومايســــــس ، جيوتريكــــــــام

Trichoderma spp., *Gliocladium* spp., *Myrothecium* spp., *Paecilomyces* spp., and *Geotrichum* spp. والتى أظــهرت نشــاطا تضاديــا واضحا ضد معظم المسببات المرضية السابقة. وقد خضعت هــذه العــزلات المضــادة لاختبارات قياسية على الاطباق لمعرفـــة أكثرهــا كفــاءة ضـــد المســببات المرضيــة المختــبرة وتبيــن منــها أن العــــزلات *Trichoderma harzianum*, (T.1), *Gliocladium virens*, (G.1), *Pseudomonas fluorescens* (B. 35), *Bacillus subtilis* (B. 5), and *Actenomycete* sp. (Act.1) هـــى أكــثر هــذه العزلات كفاءة.

- بدارسة تأثير إضافة راشح هذه الكائنات الحية إلى البيئة قبل تلقيحها بالمسببات المرضيــة المختبرة تبين أن لهذا الراشح تأثير مثبط على نمو الفطريات المرضية المختبرة ويــزداد هذا التثبيط بزيادة تركيز الراشح لأى من هذه العزلات المضادة وكان أكثر الكائنات الحية تأثيرا *T. harzianum* يليه *G. virens* عند تركيز ٥٠% ومـــن البكتريــا أظــهرت *P. fluorescens* أفضل النتائج.
- بتحليل راشح كل من الفطرين *T. harzianum* و *G. virens* لوحظ وجـــود المضــاد الحيوى Gliotoxin كأحد المنتجات الايضية لهذين الفطرين والذى أظهر كفاءة عالية فى تثبيط نمو الفطريات المرضية المختبرة عند إضافته فى ثقوب بالبيئة التـــى تـــزرع بـــها المسبب المرضى.
- كذلك لوحظ وجود مواد فينولية فى راشح كل الكائنات الحية المختبرة كمواد أيضية ثانوية لهذه العزلات المضادة والتى لها تأثير غير مباشر فى تثبيط المسببات المرضية المختــبرة وكانت كمية المواد الفينولية الكلية المقدرة أعلى ما يكون فـــى حالـــة *P. fluorescens* (٠,٢٣٣%) و *Actenomycete* Sp. (٠,١٧٣%) كمضادات بكتيريـــة و *G. virens* (٠,٠٨٤%) كمضاد فطرى.
- بعد أن ثبت كفاءة العزلات المضادة للفطريات (T.1, G.1, P.35, B.5 and Act.1) على الاطباق كاكثر العزلات المضادة كفاءة ضد الفطريات المرضية تم أختبار كفاءتها فى تجـــارب الأصـــص. فقـــد أدت معاملـــة التربـــة الملقحـــة بـــأى مـــن المســـببات المرضيـــة لنباتـــات الطمـــاطم فيوزاريـــوم اكسيســـورم ، ورايزوكتونيـــا ســـولانى *Fusarium oxysporum f.* sp. *lycopersici* and *Rhizoctonia solani*

بالعزلات T.1 (٣% من وزن التربة) ، G.1 (بمعدل ٢% مـــن وزن التربــة) ، P.35 (بمعدل ١٠ ^٨ خلية/جم تربة) أو بالعزلة B.5 (بمعدل ١٠ ^٨ خليـــة/جــم تربـــة) و Act.1 (بمعدل ١٠ ^٧ وحدة تكاثرية/جم تربة) عند الزراعة إلى تحقيق مستويات عالية المعنويـــة من الوقاية ضد أى من المسببات المرضية المختبرة والتى تقـــترب كثـيرا ممـــا يحققـه استعمال المبيد الفطرى بينوميل.

- وجدت نفس النتائج فى حالة استعمال نفس العزلات المضادة بنفس المعدلات فى معاملـــة التربة الملوثة بالمسببات المرضية *F. solani, R. solani, P. aphanidermatum* فيوزاريوم سولانى ، رايزوكتونيا سولانى بيثيوم افانيدرماتوم والتى تصيب نباتات الفلفـل حيث أنها حققت حماية كبيرة للنباتات بتقليل ظهور المرض بما يعادل ما يحققه اســتعمال الجرعة الموصى بها من المبيد الفطرى البيوميل تقريبا.
- أدت المعاملة بهذه العزلات المضادة بالمعدلات السابقة إلى زيادة معنويـــة فـــى غالبيـة الصفات الخضرية للنباتين وتحسين نمو النباتات عنها عند استعمال المبيد الفطرى وكـانت أحسن المعاملات هى *T. harzianum* يليها *G. virnes* ومـــن المضـــادات البكتيريـــا *P. fluorescens*.
- هذه النتائج التى تجمعت خلال هذه الدراسة قد تؤدى إلى التوصيـــة باســتخدام العـــزلات *T. harzianum* (T.1), *G. virens* (G.1), *P. fluorescens* (P.35), *B. subtilis* (B.5) and *Actenomycete* sp. (Act.1) ترايكودرمـــا هارزيـــانم ، جليوكلاديوم فيرنز ، سيدوموناس فلورسنس ، باسيللس ساتللس ، واكتينوميسيتس لتحقيـق مكافحة حيوية ناجحة ضد المسببات المرضية المحمولة بالتربة والتى تهاجم نباتات العائلة الباذنجانية خاصة الطماطم والفلفل وتشجيع زيادة نمو كلا النباتين.

بسم الله الرحمن الرحيم

دراسات على بعض أمراض أعفان الجذور التى تصيب بعض نباتات العائلة الباذنجانية

رسالة مقدمة من
سحر متولى حسن حمود
بكالوريوس العلوم الزراعية ــ شعبة أمراض نبات
كلية الزراعة بكفرالشيخ ــ جامعة طنطا ١٩٩٤م.

للحصول على

درجة الماجستير فى العلوم الزراعية

أمراض نبات

قسم النبات الزراعى
كلية الزراعة بكفرالشيخ
جامعة طنطا

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