CZECH MYCOL. 56 (3–4), 2004

Biological control of two phytopathogenic fungal species isolated from the rhizoplane of soybean (Glycine max)

MOHAMED HASHEM

Department of Botany, Faculty of Science, Assiut University, Assiut, Egypt
mhashem2000eg@yahoo.com

Hashem M. (2004): Biological control of two phytopathogenic fungal species isolated from the rhizoplane of soybean (Glycine max). – Czech Mycol. 56: 223–238

Two hundred isolates representing 31 fungal species (20 genera) were recovered from soybean roots. Samples were collected from 12 localities at 3 different growth stages of the crop. The most dominant species were *Aspergillus flavus, Fusarium oxysporum, Fusarium solani* (*Nectria haematococca*), *Macrophomina phaseolina* and *Rhizoctonia solani*. Pathogenicity tests have proved the ability of *Macrophomina phaseolina* and *Rhizoctonia solani* to infect soybean roots and produce the symptoms of damping-off and root-rot diseases. The efficacy of three antagonists (*Trichoderma harzianum, Epicoccum nigrum* and *Paecilomyces lilacinus*) as well as two organic compounds (Strom and F-760) was evaluated as to their control of pathogenic fungi. Biocontrol fungi significantly suppressed *Macrophomina phaseolina* and *Rhizoctonia solani* in vitro and in vivo. *Epicoccum nigrum* and *Paecilomyces lilacinus* suppressed the growth of the pathogens by producing an inhibition zone while *Trichoderma harzianum* suppressed them by overgrowing. Strom and F-760 showed lower reduction effect of diseases in comparison with the antagonists.

Keywords: biological control, soybean, *Macrophomina phaseolina, Rhizoctonia solani*

Hashem M. (2004): Biologická kontrola dvou fytopatogenních hub izolovaných z povrchu kořenů sóji (Glycine max). – Czech Mycol. 56: 223–238

Z kořenů sóji bylo získáno 200 izolátů reprezentujících 31 druhů hub z 20 rodů. Vzorky byly získány z 12 lokalit a ze 3 různých růstových stádií. Dominantními druhy byly *Aspergillus flavus, Fusarium oxysporum, Fusarium solani* (*Nectria haematococca*), *Macrophomina phaseolina* a *Rhizoctonia solani*. Testy patogenicity prokázaly schopnost druhů *Macrophomina phaseolina* a *Rhizoctonia solani* infikovat kořeny sóji a vyvolávat příznaky padání klíčních rostlin a kořenové hniloby. Byla hodnocena schopnost tří antagonistických druhů (*Trichoderma harzianum, Epicoccum nigrum* a *Paecilomyces lilacinus*) a dvou organických fungicidů (Strom a F-760) potlačovat patogenní houby. Antagonistické houby významně potlačovaly druhy *Macrophomina phaseolina* a *Rhizoctonia solani* jak in vitro, tak in vivo. *Epicoccum nigrum* a *Paecilomyces lilacinus* potlačovaly růst patogenů vytvořením inhibiční zóny, zatímco *Trichoderma harzianum* přerůstáním. Fungicidy Strom a F-760 vykazovaly menší potlačení chorob ve srovnání s antagonistickými houbami.

INTRODUCTION

Modern agriculture has tended to apply ever-larger amounts of fungicides to attain the yield potential of crops. This leads inevitably to disturbances in the biological balance and has caused severe disease outbreaks, environmental

pollution, and to increasing amounts of toxic chemicals in food chains. Nowadays, there are propelling constraints urging to look for alternative control methods in crop production. This has led to intensified research on biological control of plant diseases (Hazarika and Das 1998, El-Naggar et al. 2001).

Soybean is an important oil crop in Egypt as well as elsewhere in the world. It is attacked by many plant pathogens. *Rhizoctonia solani* and *Macrophomina phaseolina* are involved in many diseases of this crop such as damping-off, root-rot, stem-rot and charcoal rot (Vyas 1994, Ehteshmaul-Haque and Ghaffar 1995, Vallone 1998, Datta et al. 2000).

Recently, biological control of *Rhizoctonia solani* and *Macrophomina phaseolina* has intensively been studied by many researchers throughout the world (Das and Dutta 1999, Zheng and Sinclair 2000). Application of *Trichoderma* spp. as a soil drench or seed treatment to reduce the severity of soybean root diseases has received growing attention in research (Killebrew et al. 1993, Das and Dutta 1999, Izhar et al. 1999, Datta et al. 2000). Efficiency of some other antagonists e.g. *Paecilomyces lilacinus*, *Gliocladium roseum*, *Gliocladium virens*, *Aspergillus* spp. and *Penicillium* spp. in control of both *Rhizoctonia solani* and *Macrophomina phaseolina* is mentioned by many authors (Ehteshmaul-Haque et al. 1992, Siddiqui et al. 2000).

In Egypt, biological control of such organisms was studied by a few researchers (Yehia et al. 1994, Aziz et al. 1997, El-Shawadfy 1997). Thus, the present work aimed to investigate safe, economic and biological control for the management of soybean root diseases caused by *Rhizoctonia solani* and *Macrophomina phaseolina*.

MATERIALS AND METHODS

Collection of samples

Soybean root samples were collected from 12 different localities in Assiut Governorate (Upper Egypt) during April-August 2001. From each field, 3 samples were taken at three different stages [seedling (20–25 days), flowering (50–60 days) and mature (80–90 days)] of the crop, 108 samples in total. Mean disease rating (MDR) of the collected root samples was estimated with a disease index using the following formula:

$MDR = \Sigma\ (ab)/n$, where

$\Sigma\ (ab)$ is the sum of plants,

a is the degree to which the plant is affected,

b is the number of plants which has the same degree,

n is total number of diseased plants.

To detect the different degrees (to which the plant is affected), roots were classified into 6 categories:

1 = 0–<5 %; 2 = 5–<15 %; 3 = 15–<25 %; 4 = 25–<50 %; 5 = 51–<75 % and 6 = 75–100 % discoloration of roots.

Isolation of rhizoplane fungi

Soybean roots were washed in running tap water and were cut into 1-cm segments. The segments were divided into two groups. The first group was washed with sterilised distilled water 3 times, dried and inserted on the surface of potato dextrose agar (PDA) medium amended with streptomycin (60 $\mu g.ml^{-1}$). The second group was surface sterilised by soaking in 3 % solution of sodium hypochlorite (NaOCl) for 2 min., then washed 3 times with sterilised distilled water, and inoculated into another group of plates (9 cm in diameter) containing the same medium. In each Petri dish five segments were placed (10 plates per sample were used in each case) and incubated at 28 ± 2 °C for 5–7 days, then examined for fungal analysis. The fungi were examined and identified using relevant references (Raper and Thom 1949, Raper and Fennell 1965, Rifai 1969, Ellis 1976, Booth 1977, Domsch et al. 1980, Moubasher 1993).

Pathogenicity test

Pathogenicity of *Rhizoctonia solani* and *Macrophomina phaseolina* was determined on soybean plants under greenhouse conditions. Inocula of each isolate were prepared by growing in 500 ml glass bottles containing sterilised barley grain medium at 28 ± 2 °C for 21 days (modified from Soliman et al. 1993). The inoculum of each fungus was mixed thoroughly with clay soil at a rate of 2 % (w/w) and then placed in pots (20 cm in diameter). Ten surface disinfected seeds were sown in each pot. Non-infested soil was mixed with 2 % (w/w) sterilised barley grains and was used as control. Plants were watered when necessary. Three replicates were used in each treatment. Percentages of pre- and post-emergency of damping-off of seedlings were estimated after 2 weeks from planting. Twenty-five days after cultivation, percentage of survived plants was determined and then the seedlings were uprooted to estimate the mean disease rating (MDR) of root-rot in them. To verify the infection of soybean roots with the two pathogens, *Rhizoctonia solani* and *Macrophomina phaseolina* were reisolated from the infected plants.

Preliminary antagonism test

Three antagonistic fungi, viz. *Trichoderma harzianum*, *Epicoccum nigrum* and *Paecilomyces lilacinus*, were isolated from the soybean rhizoplane. The inhibitory effect of these antagonists on the growth rate of *Rhizoctonia solani* and *Macrophomina phaseolina* was studied by the dual culture method in Petri dishes according to the method described by Hazarika and Das (1998). After 5 days of incubation at 28 ± 2 °C on PDA medium, the radial growth of the pathogen's colonies in dual culture was measured and the percentage of inhibition was calculated compared to the control. Also, the inhibition zone was estimated and mycoparasitism detected visually and by light microscope.

Tested compounds

The effect of two organic compounds Strom and Fenor (F-760) on mycelial growth of both pathogens (*Rhizoctonia solani* and *Macrophomina phaseolina*) was studied in Petri dishes. Strom is a derivative of blood protein of fibrinogen, albumin and globulin with sodium cellulose glycolic acid (Schkalikov et al. 1994). Fenor (F-760) is a quaternary ammonium salt (4-aminobenzoate-2-hydroxypropyl, triethylammonia), (Schkalikov et al. 1994). The recommended dose of each compound (2.5 % for Strom and 1.4 % for F-760) was thoroughly mixed in sterilised PDA medium separately, and poured into Petri dishes (three replicates for each treatment). Mycelial disks of 4-day old cultures of the pathogens (5 mm in diameter) were placed in the middle of the plates. Plates containing PDA were only inoculated with the same tested fungi and served as control. The diameters of the colonies were measured after 1, 2, 3 and 4 days.

Pot experiments

The inoculum of antagonists and pathogens was prepared as mentioned above (under Pathogenicity test). Inocula of both antagonist and pathogen were mixed with soil (2 % w/w of each). The control treatment was mixed with 4 % (w/w) of sterilised barley seeds. In the case of Strom and F-760, the inoculum of the pathogens only was added and the seeds of soybean were dressed in suspensions of these compounds containing the recommended dose of each compound separately, before planting. Percentages of pre- and post-emergence damping-off of seedlings, survived plants, mean disease rating (MDR) of root-rot disease as well as fresh and dry weights of plants (30 days old) were estimated. The experiments were arranged in complete randomised design and the data were analysed using LSD at 5 %.

Tab. 1. Mean disease rating (MDR) of collected plants from different localities.

Locality	Plant growth stages		
	Seedling	Flowering	Mature
1	1.0	2.0	3.1
2	1.7	2.7	3.3
3	1.7	2.0	2.6
4	2.3	3.3	3.5
5	2.1	2.7	3.2
6	2.2	3.0	4.2
7	1.7	3.2	4.1
8	2.3	3.0	4.5
9	2.1	2.5	4.1
10	1.7	3.2	5.0
11	2.2	2.6	4.2
12	1.6	2.3	3.3

RESULTS

The mean disease rating (MDR) of root-rot of collected samples from different localities was estimated and is represented in Table 1. Generally, it is clear that the discoloration of the roots increased with age of the plant. The MDR fluctuated between 1.0 and 5.0. Locality 10 exhibited the highest rate (5.0), while locality 3 gained the lowest (2.6) rate at crop maturity stage.

Thirty-one fungal species belonging to 20 genera were isolated from soybean root surfaces (Table 2). In general, the most dominant genera that occurred in high frequencies throughout the three different plant stages were *Aspergillus* (63.7 %), *Fusarium* (60.7 %), *Macrophomina* (36.3 %), *Rhizopus* (15.3 %) and *Rhizoctonia* (10.5 %). In seedling stage the most dominant species were *Aspergillus flavus* (35.2 %), *Rhizopus stolonifer* (8.9 %), *F. solani* (5.8 %), *Macrophomina phaseolina* (5.4 %), *A. niger* (4.3 %) and *Fusarium moniliforme* (3.1 5 %). During the flowering stage the order of dominant species was changed and the most frequent were *Aspergillus flavus* (12.2 %), *Fusarium oxysporum* (11.8 %), *Fusarium solani* (8.0 %), *Mucor racemosus* (6.9 %), *Rhizopus stolonifer* (5.2 %), *Rhizoctoria solani* (4.0 %) and *Fusarium moniliforme* (3.5 %). At the maturity of the crop, *Macrophomina phaseolina* (26.7 %), *Fusarium oxysporum* (21.0 %), *Alternaria alternata* (20.4), *Mucor racemosus* (13.1 %) and *Fusarium solani* (5.4 %) represented the most dominant species. Other fungal species were observed in low percentage (0.1–2.3 %) during the three periods of isolation (Table 2).

Tab. 2. Percentages of fungi isolated from non-sterilised soybean roots (600 segments; 50 segments per sample).

Species	Plant growth stages			Total
	Seedling	Flowering	Mature	
Acremonium strictum W. Gams	0.0	0.8	0.9	1.7
Alternaria alternata (Fr.) Keissl.	0.1	3.2	20.4	23.7
Aspergillus	41.4	17.2	5.1	63.7
A. flavipes (Bainier et Sartory) Thom et Church	0.4	0.3	0.0	0.7
Aspergillus flavus Link	35.2	12.2	2.3	49.7
A. fumigatus Fresen.	0.3	0.2	0.0	0.5
A. niger Tiegh.	4.3	2.3	1.6	8.2
A. ochraceus G. Wilh.	0.6	0.7	0.2	1.5
A. terreus Thom	0.7	1.5	1.0	3.2
Chaetomium globosum Kunze	0.1	0.1	0.0	0.2
Cladosporium cladosporioides (Fresen.) G. A. de Vries	0.0	0.2	1.9	2.1
Cunninghamella elegans Lendn.	0.1	0.1	0.1	0.3
Curvularia lunata (Wakker) Boedijn	0.2	0.5	0.1	0.8
Drechslera	1.4	0.9	0.3	2.6
Drechslera halodes (Drechsler) Subram. et B. L. Jain	0.5	0.1	0.2	0.8
Drechslera spicifera (Bainier) Arx	0.9	0.8	0.1	1.8
Epicoccum nigrum Link	0.2	0.2	0.5	0.9
Fusarium	10.4	23.3	27.0	60.7
Fusarium moniliforme J. Sheld.	3.1	3.5	0.6	7.2
Fusarium oxysporum Schltdl.	1.5	11.8	21.0	34.3
Fusarium solani (Mart.) Sacc.	5.8	8.0	5.4	19.2
Clonostachys rosea (Link: Fr.) Schroers et al. f. *rosea*	0.0	0.5	0.2	0.7
Macrophomina phaseolina (Tassi) Goid.	5.4	4.2	26.7	36.3
Mucor racemosus Bull.	2.2	6.9	13.1	22.2
Myrothecium verrucaria (Alb. et Schwein.) Ditmar	0.0	0.1	0.1	0.2
Paecilomyces lilacinus (Thom) Samson	0.1	0.0	0.0	0.1
Penicillium	0.8	0.4	0.4	1.6
P. chrysogenum Thom	0.2	0.1	0.1	0.4
P. corylophilum Dierckx	0.2	0.1	0.1	0.4
Penicillium funiculosum Thom	0.4	0.2	0.2	0.8
Rhizoctonia solani J. G. Kühn	1.4	4.0	5.1	10.5
Rhizopus stolonifer (Ehrenb.) Lind	8.9	5.2	1.2	15.3
Stachybotrys chartarum (Ehrenb.) S. Hughes	0.1	0.0	0.1	0.2
Trichoderma harzianum Rifai	0.4	0.4	1.6	2.4
Ulocladium chartarum (Preuss) E. G. Simmons	0.1	0.2	0.2	0.5

Tab. 3. Percentages of fungi isolated from surface-sterilised soybean roots (600 segments; 50 segments per sample)

Species	Plant growth stages			Total
	Seedling	Flowering	Mature	
Alternaria alternata	5.0	8.5	15.8	29.3
Aspergillus	8.4	5.2	4.6	18.2
Aspergillus flavus	4.5	3.2	3.5	11.2
A. fumigatus	0.2	0.2	0.1	0.5
A. niger	2.1	1.5	0.5	4.1
A. ochraceus	0.5	0.1	0.2	0.8
A. terreus	1.1	2.0	0.3	3.4
Cladosporium cladosporioides	0.2	0.1	0.0	0.3
Curvularia lunata	0.8	0.5	0.4	1.7
Drechslera	3.4	7.0	5.9	16.3
Drechslera halodes	2.1	3.5	3.0	8.6
Drechslera spicifera	0.5	3.0	2.5	6.0
Epicoccum nigrum	0.1	0.2	0.2	0.5
Fusarium	11.0	22.5	49.5	83.0
Fusarium moniliforme	5.0	7.0	10.2	22.2
Fusarium oxysporum	2.5	10.5	30.5	43.5
Fusarium solani	3.5	5.0	8.5	17.0
Clonostachys rosea	0.0	0.1	0.1	0.2
Macrophomina phaseolina	8.2	15.6	40.3	64.1
Myrothecium verrucaria	0.0	0.1	0.0	0.1
Penicillium	0.5	0.5	0.6	1.6
Penicillium chrysogenum	0.3	0.4	0.5	1.2
Penicillium funiculosum	0.2	0.1	0.1	0.4
Rhizoctonia solani	5.0	8.3	8.9	22.2
Trichoderma harzianum	0.2	0.2	0.2	0.6

The data in Table 3 demonstrate that 21 fungal species were isolated from the surface sterilised roots. The most dominant species isolated in high frequency from the three stages of growth were *Macrophomina phaseolina* (64.1 %), *Fusarium oxysporum* (43.5 %), *Alternaria alternata* (29.3 %), *Fusarium moniliforme* (22.2 %), *Penicillium funiculosum* (22.2 %) *Fusarium solani* (17.0 %), *Aspergillus flavus* (11.2 %), *Drechslera halodes* (8.6 %) and *Drechslera spicifera* (6.0 %).

In seedling stage, the most dominant species were *Macrophomina phaseolina* (8.2 %), *Rhizoctonia solani* (5.0 %), *Alternaria alternata* (5.0 %), *Fusarium*

Tab. 4. Pathogenicity test of *Rhizoctonia solani* and *Macrophomina phaseolina*.

Treatments	% of damping-off		Surviving plants (%)	MDR of root-rot
	Pre-emergence	Post-emergence		
Control	6.67	3.33	90.00	1.67
Rhizoctonia solani	56.62	20.00	26.67	4.33
Macrophomina phaseolina	53.33	20.00	26.67	4.67
LSD at 5 %	11.54	6.66	9.42	1.15

Tab. 5. Inhibitory effect of antagonists on *R. solani* and *Macrophomina phaseolina* in vitro.

Treatments	Inhibition of growth (% of control)	Inhibition zone (in mm)	Mycoparasitism (overgrowth)
Rhizoctonia solani			
Epicoccum nigrum	12.28	5	–
Paecilomyces lilacinus	25.06	3.5	–
Trichoderma harzianum	43.08	0.0	+
Macrophomina phaseolina			
Epicoccum nigrum	25.76	11	–
Paecilomyces lilacinus	24.38	7.5	–
Trichoderma harzianum	50.30	0.0	+

moniliforme (5.0 %) and *Aspergillus flavus* (4.5 %). At flowering and mature stages, *Macrophomina phaseolina* occupied the first place among the isolated fungi and was detected in 15.6 % and 40.3 % of the total examined root segments, respectively. *Fusarium oxysporum*, *Fusarium moniliforme*, *Rhizoctonia solani*, *Alternaria alternata*, and *Drechslera halodes* were the most dominant species following *Macrophomina phaseolina*. The percentage of these fungi collectively, was 42.8 % at the flowering stage and 76.9 % in the mature stage of plants. The rest of the fungal species were rarely isolated (Table 3).

The results in Table 4 proved the ability of both *Rhizoctonia solani* and *Macrophomina phaseolina* to infect seedlings of soybean and cause pre- and post-emergence damping-off as well as root-rot disease. They reduced the percentage of survived plants to 26.33 % and 26.67 % and increased the MDR to 4.33 and 4.67, respectively.

The test of Strom and F-760 in the of control of *Rhizoctonia solani* and *Macrophomina phaseolina* was carried out in Petri dishes and the obtained results are represented in Figs. 1, 2 and 3. It is clear that F-760 is more effective than

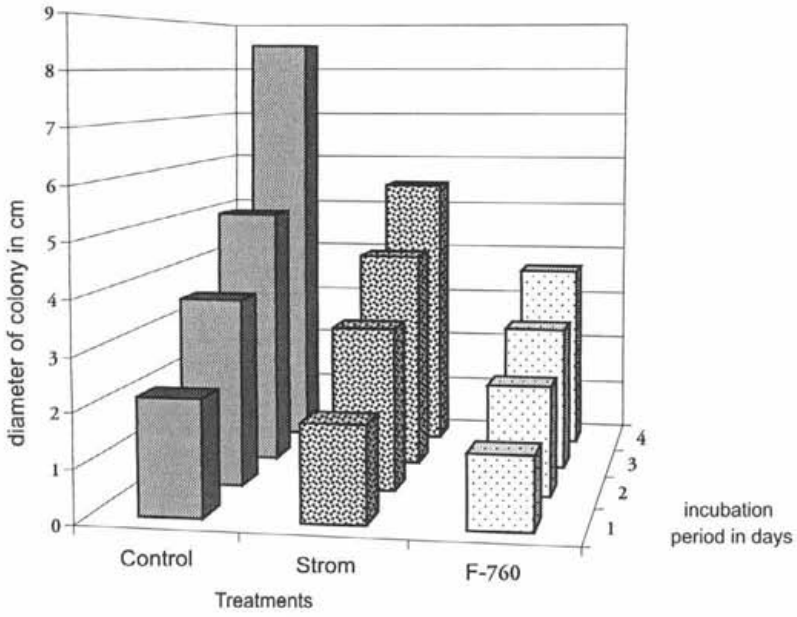

Fig. 1. Effect of Strom and F-760 on the mycelial growth of *Rhizoctonia solani*.

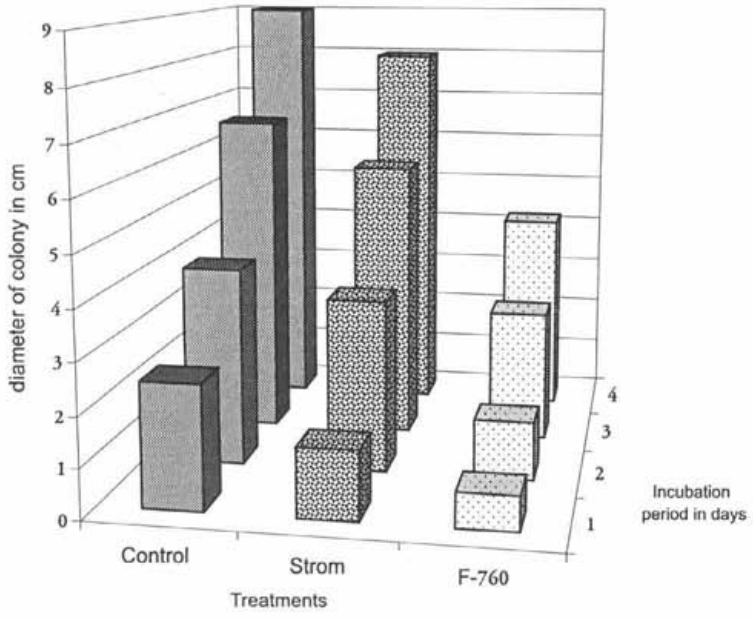

Fig. 2. Effect of Strom and F-760 on the mycelial growth of *Macrophomina phaseolina*.

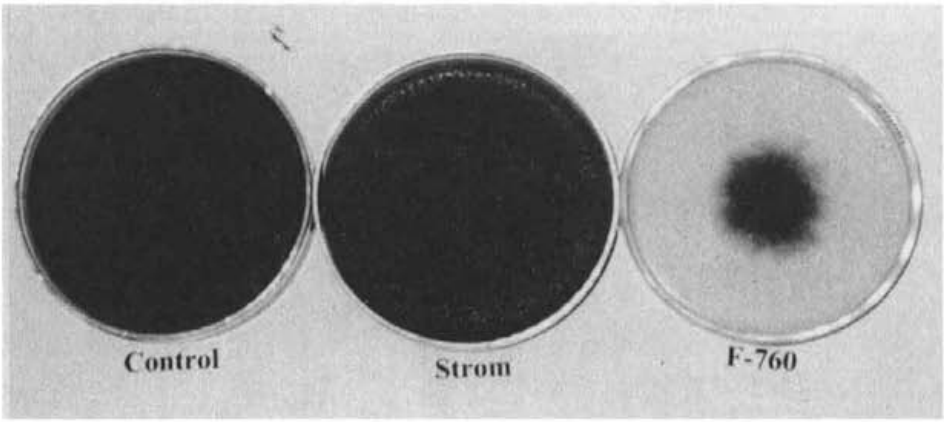

Fig. 3. Inhibition of mycelial growth of *Macrophomina phaseolina by* Strom and F-760.

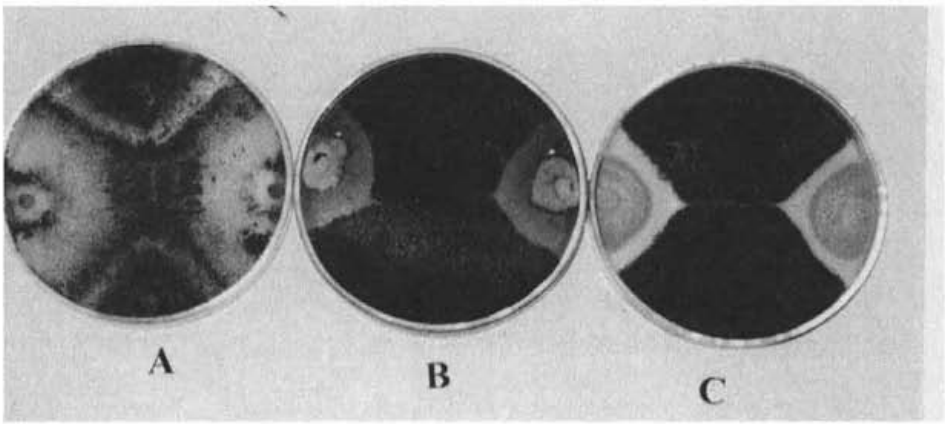

Fig. 4. Antagonist's inhibition of the growth of *Macrophomina phaseolina* by overgrowth of *Trichoderma harzianum* (A) and production of inhibition zone by *Epicoccum nigrum* (B) and *Paecilomyces lilacinus* (C).

Strom in the inhibition of the mycelial growth of the two pathogens. F-760 and Strom showed a pronounced inhibitory effect of the mycelial growth of *Rhizoctonia solani* (56.9 % and 35.3 % of the control, respectively) at the end of the incubation period (Fig. 1). *Macrophomina phaseolina* was affected by the two tested compounds all through the incubation period (Fig. 2 and 3). At the end of the experiment, this inhibition was 53.3 % by F-760 and 11.4 % in the case of Strom in comparison with the control.

The antagonistic effect of *Epicoccum nigrum*, *Paecilomyces lilacinus* and *Trichoderma harzianum* against *Rhizoctonia solani* and *Macrophomina phaseolina*

Tab. 6. Effect of antagonists and compounds on *Rhizoctonia solani* under greenhouse conditions.

Treatments	Damping off (%)		Surviving plants (%)	MDR of root-rot		Weight of seedlings	
	Pre-emergence	Post-emergence		As seedling	At maturity	Fresh	Dry
Control	10.00	3.33	86.67	1.67	2.33	2.83	0.62
Rhizoctonia solani	56.67	16.67	26.67	4.33	4.67	2.52	0.45
Rhizoctonia solani + *Epicoccum nigrum*	26.67	0.00	73.33	2.33	2.67	3.07	0.73
Rhizoctonia solani + *Trichoderma harzianum*	30.00	6.67	63.33	2.67	2.67	2.97	0.70
Rhizoctonia solani + *Paecilomyces lilacinus*	30.00	6.67	63.33	2.67	3.00	2.95	0.65
Rhizoctonia solani + Strom	30.00	16.67	53.33	3.33	3.33	2.80	0.60
Rhizoctonia solani + F-760	26.67	13.33	56.67	3.33	3.33	2.81	0.56
LSD at 5 %	9.36	9.36	10.11	1.01	0.94	0.15	0.08

was studied in the laboratory as a preliminary test. Table 5 and Fig. 4 show that *Trichoderma harzianum* exerted the highest inhibitory effect on the mycelial growth of the pathogens (it reduced the growth of *Rhizoctonia solani* and *Macrophomina phaseolina* by 43.8 % and 50.3 % of the control, respectively). The inhibition of the pathogens by *Trichoderma harzianum* was related to its ability to overgrow them (mycoparasitism), whereas *Epicoccum nigrum* and *Paecilomyces lilacinus* reduced the growth of *Rhizoctonia solani* and *Macrophomina phaseolina* by production of an inhibition zone. The width of the inhibition zone produced by *Epicoccum nigrum* was 5 and 11 mm against *Rhizoctonia solani* and *Macrophomina phaseolina,* respectively. *Paecilomyces lilacinus* exerted 3.5 and 7.5 mm wide inhibition zones against the same pathogens in the same order.

In pot experiments, the effect of the antagonists and the organic compounds on *Rhizoctonia solani* and *Macrophomina phaseolina* was studied under greenhouse conditions. Application of antagonists as soil treatment and organic compounds as seed treatment significantly reduced the pre- and post-emergence damping-off and root-rot diseases caused by *Rhizoctonia solani*. The best result was achieved by *Epicoccum nigrum* when mixed with the pathogen. It was involved in the reduction of pre-emergence of seedlings in 26.67 % of the cases and completely eliminated the post-emergence damping-off disease (Table 6).

Tab. 7. Effect of antagonists and compounds on *Macrophomina phaseolina* under greenhouse conditions.

Treatments	Damping off (%)		Surviving plants (%)	MDR of root-rot		Weight of 30 days old plant	
	Pre-emergence	Post-emergence		As seedling	At maturity	Fresh	Dry
Control	10.00	3.33	86.67	1.67	2.33	2.83	0.62
Macrophomina phaseolina	53.33	16.67	30.00	4.33	2.33	2.47	0.55
Macrophomina phaseolina + *Epicoccum nigrum*	26.67	3.33	70.00	2.33	2.33	3.08	0.84
Macrophomina phaseolina + *Trichoderma harzianum*	23.33	6.67	70.00	2.67	2.64	2.85	0.71
Macrophomina phaseolina + *Paecilomyces lilacinus*	30.00	10.00	60.00	2.67	2.67	3.10	0.81
Macrophomina phaseolina + Strom	33.33	13.33	53.33	3.67	3.67	2.96	0.72
Macrophomina phaseolina + F-760	36.67	16.67	47.67	3.67	3.67	2.92	0.67
LSD at 5 %	8.55	9.36	24.77	1.01	1.01	0.24	0.08

All other treatments (*Trichoderma harzianum, Paecilomyces lilacinus*, Strom and F-760) also significantly reduced the percentage of pre- and post-emergence damping-off compared with *Rhizoctonia solani*. The number of surviving plants was significantly increased by the application of all antagonists and organic compounds. The MDR of root-rot of soybean was reduced significantly as a result of application of antagonists or organic compounds. It ranged from 2.33 in the case of *Epicoccum nigrum* and 3.33 in case of Strom. Fresh and dry weights of seedlings increased by the application of the antagonists and organic compounds (Table 6).

The results in Table 7 show that *Macrophomina phaseolina* is probably the main causal agent of pre- and post-emergence damping-off and root-rot diseases of soybean. It was responsible for the death of 53.33 % of seedlings before germination and 16.67 % after germination. On the other hand, mixing of antagonists with these pathogens resulted in a significant reduction of both diseases. Also, these treatments improved the percentage of surviving plants as well as fresh and dry weights of seedlings. *Epicoccum nigrum* and *Trichoderma harzianum* exhibited the best effect on plants. They increased the percentage of surviving plants (70 %) and reduced the MDR to 2.33. Application of Strom and F-760 as seed treatment

also improved the health of plants and reduced the rate of disease to some extent compared with the antagonistic fungi.

DISCUSSION

In this study, thirty-one fungal species were recovered from the rhizoplane of soybean. The most dominant fungi which were isolated in high frequencies during the three periods of isolation (seedling, flowering and mature stages) were *Aspergillus flavus, Macrophomina phaseolina, Fusarium oxysporum*, *Alternaria alternata*, *Mucor racemosus, Fusarium solani, Rhizopus stolonifer, Rhizoctonia solani, A. niger* and *Fusarium moniliforme*. Most of these species, especially *Alternaria alternata*, *Fusarium moniliforme*, *Fusarium solani, Macrophomina phaseolina* and *Rhizoctonia solani* were reported as soybean root pathogen (Rupe 1991, Killebrew et al. 1993, Moubasher 1993, Aziz et al. 1997, Datta et al. 2000).

At seedling stage, *Aspergillus flavus* was the most dominant species, isolated in high frequency (35.2 % of total segments). It was recorded as a soybean root inhabiting fungus by many researchers (Tsay 1990, Dubey and Dwivedi 1988, Deb and Dutta 1991, Killebrew et al. 1993, Vyas 1994). Tsay (1990) reported that *Aspergillus flavus* reduced the emergence and caused blight and stunting symptoms on developed soybean seedlings. On the contrary, Dubey and Dwivedi (1988) and Deb and Dutta (1991) used *Aspergillus flavus* as an antagonistic organism against soybean root pathogens. Other fungi were isolated in relatively low frequencies and reported in many cases as soil or root saprophytes (Moubasher 1993, Tseng 1995).

From the surface sterilised roots, 21 species were isolated, of which *Macrophomina phaseolina, Rhizoctonia solani*, *Alternaria alternata, Fusarium moniliforme, Aspergillus flavus* and *Fusarium oxysporum* were considered the main dominant species. Isolation of these species from the soybean rhizoplane and rhizosphere was reported by many others researchers (Dubey and Dwivedi 1988, Killebrew et al. 1993, Vyas 1994).

The pathogenicity test with *Rhizoctonia solani* and *Macrophomina phaseolina* proved their ability to cause pre- and post-emergence damping-off and root-rot diseases of soybean seedlings. These results were confirmed by findings of many researchers (Vyas 1994, Ehteshamul-Haque and Ghaffar 1995, Aziz et al. 1997, El-Shawadfy 1997, Hazarika and Das 1998, Vallone 1998, Datta et al. 2000).

The preliminary test of the effect of organic compounds (Strom and F-760) on the control of *Rhizoctonia solani* and *Macrophomina phaseolina* revealed that the efficacy of F-760 in reduction of the mycelial growth of the pathogens is greater than that of Strom. These compounds were used successfully in the control of

root-rot disease of wheat by Russian scientists (Schkalikov 1995, 1996; Schkalikov and Schekhovtcova 1994; Schkalikov et al. 1994).

In dual culture, *Trichoderma harzianum*, *Paecilomyces lilacinus* and *Epicoccum nigrum* significantly reduced the mycelial growth of *Rhizoctonia solani* and *Macrophomina phaseolina*. The ability of different species of *Trichoderma* to inhibit the growth and parasitise the mycelia of *Rhizoctonia solani* and *Macrophomina phaseolina* was demonstrated by many investigators (Yehia et al. 1994, Vyas 1994, Ehteshamul-Haque and Ghaffar 1995, Das and Dutta 1999, Datta et al. 2000). This character of *Trichoderma* spp. is correlated to their ability to coil around hyphae of the pathogens and degrade them by producing degrading enzymes, especially 1,3-glucanase, chitinase and cellulase, which play an important role in lysis of the cell wall of pathogenic fungi (Hayes et al. 1993, Pisi et al. 2001)

Application of *Paecilomyces lilacinus* to control both *Rhizoctonia solani* and *Macrophomina phaseolina* was evaluated either singly or in combination with other fungi. They were used as seed dressing or soil treatment (Ehteshamul-Haque et al. 1992, Siddiqui et al. 2000).

In this study *Epicoccum nigrum* seemed to be a promising organism to control *Macrophomina phaseolina* and *Rhizoctonia solani*, while it has received little attention in the literature (Pascual et al. 1999, Huang et al. 2000).

In pot experiments, application of antagonistic fungi as soil treatments and organic compounds as seed dressing successfully controlled the target pathogens. Antagonists as well as organic compounds significantly reduced the disease index of plants and increased the percentage of emerged and surviving plants. Also, fresh and dry weights of seedlings improved as a result of application of these biocontrol agents. These results were in agreement with published data (Ehteshamul-Haque et al. 1992, Schkalikov 1995, 1996, Schkalikov and Schekhovtcova 1994, Schkalikov et al. 1994, Siddiqui et al. 2000). Ehteshamul-Haque and Ghaffar (1995) observed more than 50 % reduction in *Macrophomina phaseolina* and *Rhizoctonia solani* infection of 30-day-old soybean seedlings as a result of application of *Trichoderma harzianum*, *Trichoderma viride, Trichoderma hamatum*, *Trichoderma koningii, Trichoderma pseudokoningii* and *Bacillus japonicum* as seed treatments.

ACKNOWLEDGEMENTS

The author is grateful to Prof. Dr. V. A. Schkalikov, Head of the Plant Pathology Department, Moscow Agricultural Academy, Russia for providing the organic compounds Strom and F-760. He also thanks Prof. Dr. M. B. Mazen and Prof. Dr. A. M. Moharram, Botany Department, Faculty of Science, Assiut University, Assiut, Egypt for great help in checking the identification of the fungal genera and species recorded in this work.

REFERENCES

AZIZ N. H., EL-FOULY M. Z., EL-ESSAWY A. A. and KHALAF M. A. (1997): Influence of bean seedling root exudates on rhizosphere colonization by Trichoderma lignorum for the control of Rhizoctonia solani. – Botany Bulletin Academy Sinica 38: 33–39.

BOOTH C. (1977): Fusarium, laboratory guide to identification of the major species. – 237 p. Kew.

DAS B. C. and DUTTA P. (1999): Biological management of stem rot of soybean caused by Rhizoctonia solani Kuhn. – Journal of the Agricultural Science Society of North-East India 12(2): 217–220.

DATTA P., DAS B. C. and HAZARIKA D. K. (2000): Integrated management of soybean stem rot. – Journal of Biological Control 14(1): 67–69.

DEB P. R. and DUTTA B. K. (1991): Studies on biological control of foot rot disease of soybean caused by Sclerotium rolfsii Sacc. – Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz 98(5): 539–546.

DOMSCH K. H., GAMS W. and ANDERSON T. H. (1980): Compendium of soil fungi, Vol. 1. – 859 p. London.

DUBEY R. C. and DWIVEDI R. S. (1988): Antagonism between Macrophomina phaseolina and some rhizosphere microfungi of soybean. – International Journal of Tropical Plant Diseases 6(1): 89–84.

EHTESHAMUL-HAQUE S. and GHAFFAR A. (1995): Effect of Bradyrhizobium japonicum and fungicides in the control of root-rot disease of soybean. – Pakistan Journal of Botany 27(1): 227–232.

EHTESHAMUL-HAQUE S., HASHMI R. Y. and GHAFFAR A. (1992): Biological control of root-rot disease of lentil. – LENS Newsletter 19(2): 43–45.

ELLIS M. B. (1976): More dematiaceous hyphomycetes. – 507 p. Kew.

EL-NAGGAR M. M. F., AHMAD F. A. M., EL-FAKHARANY I. I. and BAYOUMI O. C. (2001): Effect of some pesticide residues extracted from tomato fruits and potato tubers on albino rats. – In: Proceeding of the first conference on safe alternative of pesticides for pest management, Assiut University, Faculty of Agriculture, 28–29th Oct., p. 131–144.

EL-SHAWADFY M. M. (1997): Biological management of soil-borne pathogens and root-knot nematode complexes on soybean. – In: Proceedings of the first international workshop of Afro-Asian nematologists, Menoufiya University, Egypt, 8–13 June.

HAYES C. K., PETERBAUER C., TRONSMO A., KLEMSDAL S. and HARMAN G. E. (1993): Antifungal chitinolytic enzymes from Trichoderma harzianum and Gliocladium virens purification, characterization, biological activity and molecular cloning. – In: Muzzarelli R. A. A. (ed), Chitin Enzymology, p. 383–392, Ancona, Italy.

HAZARIKA D. K. and DAS K. K. (1998): Biological management of root-rot of French bean (Phaseolus vulgaris L.) caused by Rhizoctonia solani. – Plant Diseases Research 13(2): 101–105.

HUANG H. C., BREMER E., HYNES R. K. and ERICKSON R. S. (2000): Foliar application of fungal biocontrol agents for the control of white mold of dry bean caused by Sclerotinia sclerotiorum. – Biological Control 18(3): 270–276.

IZHAR I., SIDDIQUI I. A., EHTESHAMUL-HAQUE S. and GHAFFAR A. (1999): Multiplication of plant growth-promoting rhizobacteria for use in the control of root-rot disease of crop plants. – Pakistan Journal of Botany 31(2): 397–405.

KILLEBREW J. F., ROY K. W. and ABNEY T. S. (1993): Fusarial and other fungi on soybean seedlings and roots of older plants and interrelationships among fungi, symptoms and soil characteristics. – Canadian Journal of Plant Pathology 15(3): 139–146.

MOUBASHER A. A. (1993): Soil fungi in Qatar and other Arab countries. – 566 p. Qatar.

PASCUAL S., MELGAREJO P. and MAGAN N. (1999): Production of the fungal biocontrol agent Epicoccum nigrum by solid substrate fermentation: effect of water activity on accumulation of compatible solutes. – Mycopathologia 146(2): 83–89.

PISI A., ROBERTI R., ZAKRISSON E., FILIPPINI G., MANTOVANI W. and CESARI A. (2001): SEM investigation about hyphal relationships between some antagonistic fungi against Fusarium spp. foot rot pathogen of wheat. – Phytopath. Mediterr. 40: 37–44.

RAPER K. B. and FENNELL D. J. (1965): The genus Aspergillus. – 686 p. Baltimore.

RAPER K. B. and THOM C. (1949): A manual of Penicillium. – 875 p. Baltimore.

RIFAI E. (1969): A revision of the genus Trichoderma. – Mycologia 116: 1–56.

RUPE J. C., GBUR E. E. and MARX D. M. (1991): Cultivar responses to sudden death syndrome of soybean. – Plant Dis. 75: 47–50.

SCHKALIKOV V. A. (1995): Ecology of protection of grain crops against diseases. – Moscow Agricultural Academy's Bulletin 2: 142–147 (in Russian).

SCHKALIKOV V. A. (1996): A complex method of protection of grain crops from root-rots. – D. Sc. thesis, Moscow Agricultural Academy (in Russian).

SCHKALIKOV V. A. and SCHEKHOVTCOVA O. N. (1994): Biological control of wheat from root-rots. – Protection of Plants 11: 20 (in Russian).

SCHKALIKOV V. A., SCHEKHOVTCOVA O. N. and IBRAHIM H. H. (1994): Proteins and Strom – inducers of immunity of grain crops to root-rots. – Protection of Plants 6:12 (in Russian).

SIDDIQUI I. A., QURESHI S. A., SULTANA V., EHTESHAMUL-HAQUE S. and GHAFFAR A. (2000): Biological control of root-rot and root knot disease complex of tomato. – Plant and Soil 227(1–2): 163–169.

SOLIMAN N. H., MANSOUR M. S., IBRAHIM I. A. M. and ABADA K. A. (1993): Biological and chemical control of broad-bean.– Journal of Applied Science 8(7): 347–351.

TSAY J. G. (1990): Aspergillus seedling blight of soybean and factors affecting its development. – Plant Protection Bulletin 32: 83–194.

TSENG T. C., TU J. C. and TZEAN S. S. (1995): Mycoflora and mycotoxins in dry bean (Phaseolus vulgaris) produced in Taiwan in Ontario, Canada. – Bot. Bull. Acad. Sin. 36: 229–234.

VALLONE S. (1998): Disease management in no-tillage soybean system.– JIRCAS Working Report 13: 35–42.

VYAS S. C. (1994): Integrated biological and chemical control of dry root-rot on soybean. – Indian Journal of Mycology and Plant Pathology 24(2): 132–134.

YEHIA A. H., ABD-EL-KADER D. A., SALEM D. E. and SAYED-AHMED A. A. (1994): Biological soil treatment with Trichoderma harzianum to control brown stem rot of soybean in Egypt. – Egyptian Journal of Phytopathology 22(2): 143–157.

ZHENG X. Y. and SINCLAIR J. B. (2000): The effects of traits of Bacillus megaterium on seed and root colonization and their correlation with the suppression of Rhizoctonia root-rot of soybean. – Biological Control 45(2): 223–243.