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BIOLOGICAL CONTROL OF SOME PLANT DISEASES BY USING SECONDARY METABOLITES OF GENUS *Drechslera*

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ABSTRACT

Culture filtrates of nine *Drechslera* isolates (*D. australiensis, D. cactivora, D. cynodontis, D. ellisii, D. hawaiensis, D. maydis, D. neergaardii, D. poae and D. spicifera*) used at concentrations of 30, 50 and 70%, were evaluated *in vitro* against mycelial growth and spore germination of 8 plant pathogenic fungi (*Alternaria solani, Botrytis cinerea, Botrytis fabae, Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, Sclerotinia sclerotiorum* and *Sclerotium cepivorum*). Among the tested culture filtrates, only *D. cynodontis* culture filtrate was highly effective growth inhibitor against all tested fungi, it reducing the fungal growth from 51.1% to 86.7%, and it is the strongest inhibitors to spore germination which inhibited spore germination of all tested fungi by 92% to 98%. Chloroform extract of *Drechslera cynodontis* culture filtrates was the supreme growth inhibitor against all tested fungi, whereas it inhibited the fungal growth from 66.7% (*R. solani*) to 88.9% (*S. cepivorum*) at concentration of 30 mg/ml. Also, chloroform extract was highly effective in suppressing spore germination of all tested fungi at all concentrations. In greenhouse experiments, chloroform extract was the highest effect in controlling the damping-off disease caused by *F. solani* and *S. sclerotiorum* on bean (93.4% control). Ethyl acetate extract was the second best (80% control for *S. sclerotiorum* and 66.7% for *R. solani*).

Keywords: *Drechslera*, culture filtrates, biological control, antifungal activity, plant pathogenic fungi, bean damping-off

INTRODUCTION

The kingdom fungi comprise a highly diverse array of species. Where species diversification has been studied in detail, fungal species can be 6 times as numerous as those of flowering plants. On this basis, since approximately 270,000 flowering plants are known today, it is estimated that more than 1.5 million fungal species may exist (Carlile and Watkinson 1994; Hawksworth 2001). Among the most fascinating and important properties of fungi is their ability to produce a tremendous variety of so-called secondary metabolites that display a broad range of biological activities.

The term phytotoxins is defined as a microbial secondary metabolite which is toxic to plant, produced by the pathogen in culture and in infected plant tissues. These metabolites are produced by the invading microorganism to facilitate the penetration and colonization of the plant tissues. Based on the reactions of host plants these toxins are roughly divided into two groups: (1) the host-selective toxins, which are produced only by a few fungal species

(e.g. *Alternaria, Cochliobolus*), are toxic only to the hosts of these pathogens and show little or no toxicity to nonsussceptible plants; and (2) the non-selective toxins, which are synthesized by a lot of fungi and bacteria and cause damage not only to the host plant but also to other plant species that are not normally attacked by the pathogen in nature. In many cases, the disease symptoms caused by non-selective toxins are very similar and very often these symptoms are chlorotic lesion and/or wilt. (Heiser *et al.*, 1998).

Most phytotoxins probably have not yet been discovered let alone chemically and biologically identified. The sources of variability are numerous. For example, species belonging to the same genus often are able to produce a wide variety of metabolites. *Alternaria*, *Claviceps* or *Fusarium* species produce at least 100 different toxic metabolites. Toxins belonging to the same structural group can be produced by different microorganisms belonging to many different genera. This is the case for trichothecenes, a family of mammalian toxic tetracyclic sesquiterpenoid substances (more than 50) produced by different genera, including *Fusarium* (producing at least 25 different trichothecenes), *Myrothecium* (producing roridins and verrucarins), *Stachybotrys* (satratoxins) and *Trichoderma* (trichodermins); or destruxins, (Pedras *et al.*, 2002) a family of cyclic peptide toxins known for their insecticidal and herbicidal properties, produced in many different variants by the entomopathogenic fungus *Metarhizium anisopliae* and by three unrelated plant pathogenic fungi, *Alternaria brassicae, Trichothecium roseum* and *Ophiosphaerella herpotricha*. Ophiobolins, a group of sesterterpenoids that includes at least 23 biogenic analogs, are produced by phytopathogenic species of genera such as *Drechslera* (Au *et al.*, 2000).

Phytotoxins may be useful tools for the study of particular physiological processes occurring in plants, since a better understanding of their mode of action can reveal new and unexpected physiological aspects and biochemical mechanisms. It was shown that the virulence (severity of disease symptoms) of plant pathogens, and sometimes their pathogenicity (ability to produce disease symptoms) may depend on their ability to produce phytotoxins. Studies on the mechanisms of toxin production can lead to a better understanding of the role of phytotoxins in disease development (Graniti, 1991).

The objectives of this study are: 1) Extraction of fungitoxic metabolites from culture filtrates of genus *Drechslera*, 2) Effect of culture filtrates and organic solvent extracts on mycelial growth and spore germination of various phytopathogenic fungi, 3) Determination of the minimum inhibitory concentration (MIC), 4) The use of natural products isolated from fungi for the control of fungal diseases in plant alternative to synthetic fungicides under greenhouse conditions.

MATERIALS AND METHODS

Drechslera isolates

Drechslera isolates (*D. australiensis, D. cactivora, D. cynodontis, D. ellisii, D. hawaiensis, D. neergaardii, D. poae* and *D. spicifera*) were purchased from the Centraalbureau voor Schimmelcultures (CBS), Utrecht, The Netherlands and Assiut University Mycological Centre (AUMC), Egypt.

Plant pathogens

Botrytis fabae, Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, Sclerotinia sclerotiorum and *Sclerotium cepivorum* were isolated from different crops on potato dextrose agar (PDA) and Czapek agar media. The plates were incubated at 26 ± 2°C or 20 ± 2°C for 7 days. Cultures were purified using single spore or hyphal tip techniques. Pure cultures were identified by mycologists from the Assiut University Mycological Centre (AUMC), Egypt. The fungal cultures were maintained on half-strength potato dextrose agar slants in small vials under mineral oil at 4°C.

Fungal strains of *Alternaria solani* and *Botrytis cinerea* were obtained from the Plant Pathology Institute, Agricultural Research Center, Giza, Egypt.

Production of culture filtrates

For toxin production, small pieces from mycelial mats of 10-day-old cultures of each isolate of Drechslera, grown on PDA petri dishes, were transferred to 500 ml flasks containing 200 ml of modified M-1-D medium. This medium consisted of 0.28 g Calcium nitrate, 0.08 g Potassium nitrate, 0.06 g Potassium chloride, 0.36 g Magnesium sulfate, 0.02 g Sodium dihydrogen phosphate.H_20, 30 g Sucrose, 5 g Ammonium tartrate, 2 mg Ferric chloride, 5 mg Maganese sulfate, 2.5 mg Zinc sulfate.$7H_20$, 1.4 mg Boric acid, 0.7 mg Potassium iodide and 1liter of Distilled water. The pH of the medium was adjusted to 5.5 using 1 M HCl, then adds 0.25 g yeast extract and was autoclaved at 120°C for 15 min. The cultures were incubated under static conditions at 27 ± 2°C in the dark for 4 weeks (Pinkerton and Strobel 1976). The culture fluid was obtained by filtrating through three layers of cheesecloth, and then two layers of the Whatman No.1 filter paper (Whatman International Ltd., Maidstone, England).

Extraction of the metabolites

Only, culture filtrates of *D. cynodontis* were concentrated to 10% of their original volume by using freeze drying. The concentrated filtrate was extracted with chloroform followed by ethyl acetate, then *n*-butanol using separating funnel (3 extractions each using 0.3 volume of organic solvent per volume of filtrate). The chloroform, ethyl acetate and *n*-butanol extracts were filtered over anhydrous Na_2SO4. All the organic fractions separated by solvent extraction were subjected to dryness using rotary evaporator to remove any traces of solvents and to obtain the final residues. The residues were weighed, and collected in vials using methanol. Residues were named as obtained, fraction A (chloroform extract), fraction B (ethyl acetate extract) and fraction C (butanol extract).

Antifungal activity against some important phytopathogens

The antifungal activity of culture filtrates and organic solvent extracts to tested fungi was investigated using the following mycelial radial growth bioassay. Concentrations were prepared in the range 30 – 70% from culture filtrates. The test was carried out by growing each fungal species in petri dishes containing 20 ml of potato dextrose agar (PDA) amended with concentrations of 30, 50 and 70% of each culture filtrates. Concentrations 10, 20 and 30 mg/ml from chloroform extract, ethyl acetate extract and butanol extract were prepared similarly (control plates containing only DMSO). The centre of each PDA plate was inoculated with one fungal disc (5 mm diameter) from edges of fungal solid cultures of the respective fungus. The plates were incubated at 24 ± 2°C for 1-2 weeks, depending on the fungal species. The antifungal activity was evaluated by measuring the diameter of test and control colonies in millimeter. Each assay was repeated at least three times. The percentage of inhibition was calculated on the basis of growth in the control plates using the expression:

$$\% \text{ Mycelial inhibition} = (X - Y / X) \times 100$$

Where X and Y are the average diameters of mycelial colonies in control and treated fungi sets.

Effects on spore germination of various phytopathogenic fungi

The assays were carried out with *A. solani, B. cinerea, B. fabae, F. oxysporum* and *F. Solani*, as test fungi. Cultures of the fungi were grown on PDA and/or potato carrot agar (PCA) media for 7 – 10 days at 25 ± 2°C until well sporulated except *A. solani*. The spores were harvested by adding 10 ml of sterile water and aseptically dislodging the spores with a sterile inoculating loop. Spores suspensions were aseptically filtered through sterile cheesecloth to remove mycelial debris. Culture filtrates of each *Drechslera* isolate were diluted in sterile distilled water to produce twofold serial concentrations 30, 50 and 70%, and 1 ml portions of each concentration were add to test tubes. Spores suspended in distilled water were diluted in potato dextrose broth to produce the inoculum of 1.0×10^5 spores/ml by using haemocytometer slide. Portions of 1 ml of a particular fungal spore suspension were added to the tubes that contained culture filtrates or distilled water (control). These tubes were then incubated at 24 ± 2°C for 24 hours. The percentage of spore germination was calculated in 100 spores from 5 microscopic fields. Three tubes were prepared for each treatment and the data was recorded as the mean of three replicates. Similar experiment was carried out separately with chloroform extract, ethyl acetate extract and butanol extract (concentrations 10, 20 and 30 mg/ml), control tubes containing only DMSO.

Sporulation was induced in *A. solani* by growing it in culture plates of PMDA medium (15 g instant mashed potato mix, 15 g dextrose, 23 g agar and 1.5 l water) for 10 to 14 days at room temperature under constant or diurnal light, then cutting the culture medium into 4-cm strips and placing them in 250 ml sterile water in flasks. Vigorously shake the flasks for 1 min, let stand for 10 min, then spread 1.5 ml of the fluid on fresh plates of this

medium and incubate under constant fluorescent light at 20°C or in diurnal light if above 23°C.

Transferring small block from a 2-day-old PDA colony of *A. solani* to corn meal agar medium and incubating under light for 4 hours, then in the dark at 18°C induces sporulation within 12 hours (Dhingra and Sinclair 1995).

Determination of the minimum inhibitory concentrations MIC

The MIC for fungal growth

The MIC was determined as the lowest concentration of culture filtrates and organic solvent extracts that completely inhibited visible fungal growth.

The MIC for spore germination

The MIC was determined as the lowest concentration of culture filtrates and organic solvent extracts that induced no germination of fungal spores or inhibited 90% or more of spore germination.

Pathogenicity tests

Cultures of fungi (*F. oxysporum, F. solani, R. solani* and *S. sclerotiorum*) were grown 5 - 10 days on PDA, and a 5 to 10 mm block of agar with hyphal tips was transferred to 150 ml of 3% cornmeal sand in 250 ml Erlenmeyer flasks. Cultures were grown 2 to 3 weeks on cornmeal sand at 22 ± 2°C. Soil was infested separately with fungal cultures (0.1 to 1% vol / vol). Controls were non infested soil. A randomized complete block design with three replications was used in all experiments. Seeds of cowpea, pea, squash, bean and pepper were surface-sterilized with 3% sodium hypochlorite solution for 3 minutes, then washed with tap water. Five seeds were sown in each pot and the pre and post emergence damping off were recorded 20 and 40 days after planting, respectively.

Effect on the development of the damping-off disease

The soil used in this experiment was sterilized before being infested with fungal isolates (*F. oxysporum, F. solani, R. solani* and *S. sclerotiorum*) at the rate of 1% by weight. The inoculums of the pathogen was prepared by growing each tested fungus on 3% cornmeal sand medium for 2 to 3 weeks at 22 ± 2°C. Surface sterilized seeds of bean were soaked for 1 - 2 hours in crude organic solvent extracts (chloroform extract, ethyl acetate extract and butanol extract). Seeds were sown at the rate of 5 seeds/pot, and three pots were used for each treatment.

Disease assessment

Percentage of pre- emergence damping off was determined after 20 days as:

$$\% \text{ pre- emergence} = \frac{\text{No. of ungerminated seeds / pot}}{\text{No. of sown seeds / pot}} \times 100$$

Percentage of post- emergence damping off was determined after 40 days as:

$$\% \text{ post- emergence} = \frac{\text{No. of died seedlings / pot}}{\text{No. of survival seedlings / pot}} \times 100$$

RESULTS

Antifungal activity of culture filtrates of *Drechslera* isolates on the mycelial growth

Data in Tables (1 and 2) show that among the tested culture filtrates, only *D. cynodontis* culture filtrate was highly effective growth inhibitor against all tested fungi, it reducing the fungal growth from 51.1% (on *R. solani*) to 86.7% (on *Sclerotium cepivorum*) at concentration of 70%. Also, *D. ellisii* culture filtrates exhibited high inhibition on the fungal growth of the tested fungi from 22.2% (on *R. solani*) to 77.8% (on *A. solani*) at the same concentration.

Antifungal activity of culture filtrates of *Drechslera* isolates on spore germination

The strongest inhibitors to spore germination among the tested culture filtrates were *D. cynodontis*, which inhibited spore germination of all tested fungi by 92% to 98%. The culture filtrates of *D. ellisii* were the second best in this regard where it reduced spore germination by 80% (*B. cinerea*) to 94% (*A. solani*) at concentration of 70%. The culture filtrates of *D. maydis* were the third best in terms of suppressing germination of fungal spores. It strongly inhibited spore germination of all tested fungi by 71 - 82%, Table (3).

Determination of the minimum inhibitory concentrations MIC

The MIC for fungal growth

Even using the maximum concentration (70%) of all culture filtrates of *Drechslera* isolates did not inhibit completely the growth of tested fungi. All the culture filtrates exhibited higher values of MIC (>70%).

The MIC for spore germination

Data in Table (4) show that the culture filtrates of *D. cynodontis* had the highest level of inhibition to spore germination of *A. solani* when used at concentration of 50%, and inhibited 90% or more of spore germination of *B. cinerea, B. fabae, F. oxysporum* and *F. solani* at concentration of 70%. This was followed by culture filtrates of *D. ellisii*. It inhibited the spore germination of *A. solani, F. oxysporum* and *F. solani* at concentration of 70%, while recorded higher values of MIC (>70%) for spore germination of *B. cinerea* and *B. fabae*. The other culture filtrates of *Drechslera* isolates had less inhibitory effect on spore germination of all tested fungi (MIC >70%).

Table (1): Antifungal activity of culture filtrates of *Drechslera* isolates on the mycelial growth of tested fungi

Fungi	%Inhibition											
	Alternaria solani			*Botrytis cinerea*			*Botrytis fabae*			*Fusarium oxysporum*		
	30%	50%	70%	30%	50%	70%	30%	50%	70%	30%	50%	70%
D. australiensis	27.8	61.1	75.6	33.3	44.4	55.6	0	11.1	33.3	16.7	33.3	44.4
D. cactivora	33.3	50	66.7	0	0	11.1	0	11.1	22.2	16.7	38.9	50
D. cynodontis	63.3	72.2	82.2	66.7	71.1	75.6	48.9	55.6	72.2	36.7	48.9	61.1
D. ellisii	61.1	72.2	77.8	27.8	33.3	50	46.7	55.6	66.7	22.2	27.8	50
D. hawaiensis	22.2	42.2	50	0	11.1	44.4	0	2.2	15.6	16.7	22.2	44.4
D. maydis	30	38.9	60	22.2	30	55.6	11.1	20	55.6	22.2	27.8	44.4
D. neergaardii	33.3	48.9	55.6	33.3	44.4	50	11.1	50	61.1	11.1	20	33.3
D. poae	16.7	33.3	33.3	0	11.1	11.1	0	11.1	11.1	16.7	22.2	22.2
D. spicifera	11.1	17.8	22.2	0	11.1	11.1	0	11.1	11.1	11.1	22.2	22.2

Table (2): Antifungal activity of culture filtrates of *Drechslera* isolates on the mycelial growth of tested fungi

Fungi	%Inhibition											
	Fusarium solani			*Rhizoctonia solani*			*Sclerotinia sclerotiorum*			*Sclerotium cepivorum*		
	30%	50%	70%	30%	50%	70%	30%	50%	70%	30%	50%	70%
D. australiensis	33.3	44.4	50	0	5.6	11.1	0	11.1	13.3	0	33.3	55.6
D. cactivora	44.4	44.4	44.4	0	5.6	11.1	0	5.6	18.9	0	33.3	51.1
D. cynodontis	44.4	53.3	62.2	10	13.3	51.1	41.1	57.8	66.7	71.1	74.4	86.7
D. ellisii	44.4	48.9	55.6	7.8	11.1	22.2	16.7	24.4	33.3	60	64.4	70
D. hawaiensis	33.3	36.7	44.4	0	0	6.7	11.1	11.1	11.1	11.1	22.2	33.3
D. maydis	33.3	55.6	58.9	4.4	12.2	33.3	11.1	22.2	44.4	66.7	75.6	81.1
D. neergaardii	33.3	41.1	51.1	0	0	5.6	0	11.1	11.1	53.3	58.9	66.7
D. poae	27.8	27.8	33.3	0	5.6	5.6	0	6.7	12.2	0	22.2	33.3
D. spicifera	11.1	20	22.2	0	5.6	10	0	6.7	11.1	0	25.6	33.3

Table (3): Antifungal activity of culture filtrates of *Drechslera* isolates on spore germination of tested fungi

Fungi	%Inhibition														
	Alternaria solani			*Botrytis cinerea*			*Botrytis fabae*			*Fusarium oxysporum*			*Fusarium solani*		
	30%	50%	70%	30%	50%	70%	30%	50%	70%	30%	50%	70%	30%	50%	70%
D. australiensis	74	82	88	31	44	57	12	21	36	16	30	42	39	59	85
D. cactivora	61	77	80	20	30	43	18	23	39	20	31	50	30	48	80
D. cynodontis	88	95	98	76	84	94	74	83	92	74	80	92	82	89	98
D. ellisii	84	88	94	48	61	80	59	73	82	67	79	90	50	77	90
D. hawaiensis	31	48	63	23	40	56	11	19	30	23	31	48	29	50	61
D. maydis	49	61	75	34	57	71	49	63	76	60	73	81	64	73	82
D. neergaardii	28	50	68	30	48	69	25	47	59	9	15	28	20	38	56
D. poae	23	31	48	16	27	34	11	24	33	34	51	68	13	23	36
D. spicifera	11	22	34	19	21	27	10	22	30	11	27	39	51	62	74

Table (4): The MIC of culture filtrates of *Drechslera* isolates on germination of fungal spores

Fungi	*Alternaria solani*	*Botrytis cinerea*	*Botrytis fabae*	*Fusarium oxysporum*	*Fusarium solani*
D. australiensis	>70	>70	>70	>70	>70
D. cactivora	>70	>70	>70	>70	>70
D. cynodontis	50	70	70	70	70
D. ellisii	70	>70	>70	70	70
D. hawaiensis	>70	>70	>70	>70	>70
D. maydis	>70	>70	>70	>70	>70
D. neergaardii	>70	>70	>70	>70	>70
D. poae	>70	>70	>70	>70	>70
D. spicifera	>70	>70	>70	>70	>70

Antifungal activity of organic solvent extracts of *Drechslera cynodontis* culture filtrates on the growth of tested fungi

Data in Tables (5 and 6) show that chloroform extract was the supreme growth inhibitor against all tested fungi, whereas it inhibited the fungal growth from 66.7% (*R. solani*) to 88.9% (*S. cepivorum)* at concentration of 30 mg/ml. Also, ethyl acetate extract exhibited high inhibition on the fungal growth from 55.6% (*S. sclerotiorum)* to 87.7% (*B. fabae*) at this concentration, while butanol extract decreased the fungal growth by 33.3 - 70%.

Antifungal activity of organic solvent extracts of *Drechslera cynodontis* culture filtrates on spore germination of tested fungi

Data in Table (7) show that chloroform extract was highly effective in suppressing spore germination of all tested fungi at all concentrations, whereas it inhibited the germination of spores from 88% (*B. fabae*) to 99% (*A. solani*) at concentrations of 30 mg/ml. Extract of ethyl acetate highly inhibited spore germination from 70% (*B. fabae*) to 96% (*A. solani*) at the same concentration, while butanol extract induced 50% and 85% inhibition for spore germination of *F. oxysporum* and *A. solani*, respectively at the maximum concentration (30 mg/ml).

Table (5): Antifungal activity of organic solvent extracts of *Drechslera cynodontis* culture filtrates on the mycelial growth of tested fungi

Extracts	%Inhibition											
	Alternaria solani			*Botrytis cinerea*			*Botrytis fabae*			*Fusarium oxysporum*		
	10 mg/ml	20 mg/ml	30 mg/ml	10 mg/ml	20 mg/ml	30 mg/ml	10 mg/ml	20 mg/ml	30 mg/ml	10 mg/ml	20 mg/ml	30 mg/ml
Chloroform	33.3	71.1	86.7	27.8	72.2	81.1	44.4	58.9	87.7	38.9	55.6	77.8
Ethyl acetate	11.1	53.3	66.7	27.8	44.4	61.1	44.4	55.6	87.7	27.8	50	60
Butanol	11.1	22.2	51.1	22.2	33.3	50	27.8	41.1	70	11.1	20	48.9

Table (6): Antifungal activity of organic solvent extracts of *Drechslera cynodontis* culture filtrates on the mycelial growth of tested fungi

Extracts	%Inhibition											
	Fusarium solani			*Rhizoctonia solani*			*Sclerotinia sclerotiorum*			*Sclerotium cepivorum*		
	10 mg/ml	20 mg/ml	30 mg/ml	10 mg/ml	20 mg/ml	30 mg/ml	10 mg/ml	20 mg/ml	30 mg/ml	10 mg/ml	20 mg/ml	30 mg/ml
Chloroform	55.6	71.1	80	22.2	50	66.7	48.9	53.3	74.4	50	72.2	88.9
Ethyl acetate	33.3	50	70	22.2	50	60	36.7	41.1	55.6	44.4	61.1	81.1
Butanol	15.6	27.8	50	5.6	22.2	44.4	22.2	27.8	33.3	33.3	55.6	70

Table (7): Antifungal activity of organic solvent extracts of *Drechslera cynodontis* culture filtrates on spore germination of tested fungi

Extracts	%Inhibition														
	Alternaria solani			*Botrytis cinerea*			*Botrytis fabae*			*Fusarium oxysporum*			*Fusarium solani*		
	10 mg/ml	20 mg/ml	30 mg/ml	10 mg/ml	20 mg/ml	30 mg/ml	10 mg/ml	20 mg/ml	30 mg/ml	10 mg/ml	20 mg/ml	30 mg/ml	10 mg/ml	20 mg/ml	30 mg/ml
Chloroform	88	95	99	76	91	98	65	79	88	75	84	93	75	95	98
Ethyl acetate	77	84	96	50	63	80	30	53	70	60	77	90	64	77	87
Butanol	63	75	85	27	50	67	34	51	70	20	32	50	34	51	74

Determination of the minimum inhibitory concentrations MIC
The MIC for fungal growth

Data in Table (8) show that the maximum concentration (30 mg/ml) of any organic solvent extracts of *Drechslera cynodontis* culture filtrates was not sufficient for inhibiting completely the growth of tested fungi. All tested extracts recorded higher MIC values (>30 mg/ml).

Table (8): The MIC of organic solvent extracts of *Drechslera cynodontis* culture filtrates on the growth of the tested fungi

Extracts	*Alternaria solani*	*Botrytis cinerea*	*Botrytis fabae*	*Fusarium oxysporum*	*Fusarium solani*	*Rhizoctonia solani*	*Sclerotinia sclerotiorum*	*Sclerotium cepivorum*
Chloroform	>30	>30	>30	>30	>30	>30	>30	>30
Ethyl acetate	>30	>30	>30	>30	>30	>30	>30	>30
Butanol	>30	>30	>30	>30	>30	>30	>30	>30

* Concentration 30 mg/ml.

The MIC for spore germination

Data in Table (9) show that the chloroform extract was the strongest inhibitor for spore germination of *A. solani, B. cinerea* and *F. solani*, its MIC was 20 mg/ml, and 30 mg/ml for *F. oxysporum*, while MIC for *B. fabae* was >30 mg/ml. It was followed by the ethyl acetate extract, which recorded the lowest MIC against spore germination of *A. solani* and *F. oxysporum* (30 mg/ml), while MIC for *B. cinerea, B. fabae* and *F. solani* was >30 mg/ml. Butanol extract had less inhibitory effect on spore germination of tested fungi, It had high values of MIC (>30 mg/ml).

Table (9): The MIC of organic solvent extracts of *Drechslera cynodontis* culture filtrates on spore germination of the tested fungi

Extracts	*Alternaria solani*	*Botrytis cinerea*	*Botrytis fabae*	*Fusarium oxysporum*	*Fusarium solani*
Chloroform	20	20	>30	30	20
Ethyl acetate	30	>30	>30	30	>30
Butanol	>30	>30	>30	>30	>30

* Concentration 20 mg/ml.

Greenhouse Experiments
Pathogenicity tests

Pathogenicity tests proved that all tested fungi were able to cause damping-off infection on bean plants with different degrees at both pre- and post-emergence stages. Results in Table (10) show that, *F. oxysporum* and *R. solani* were the most fungi isolates caused damping-off disease to bean plants. Isolates of *F. solani* and *S. sclerotiorum* caused a highly significantly effect at pre- and post-emergence stages at the rate of 86.6 % and 6.6 %, respectively. As the least percent of survival plants were recorded with *F. oxysporum* and *R. solani* (0%) followed by 6.8% with *F. solani* and *S. sclerotiorum* under greenhouse condition. *F. solani* had a pathogenic effect on squash causing 100% pre-emergence damping-off. However, it was less effective against pepper, pea and cowpea inducing 86.6, 73.3 and 60% pre-emergence damping-off, respectively.

Table (10): Pathogenicity tests of selected fungi against some plants

Plant species	*Fusarium oxysporum*			*Fusarium solani*			*Rhizoctonia solani*			*Sclerotinia sclerotiorum*		
	Pre -	Post -	Survival	Pre -	Post -	Survival	Pre -	Post -	Survival	Pre -	Post -	Survival
Ben	100	0	0	86.6	6.6	6.8	100	0	0	86.6	6.6	6.8
Cowpea	86.6	6.6	6.8	60	13.3	26.7	100	0	0	66.6	0	33.4
Pea	40	13.3	46.7	73.3	13.3	13.4	46.6	0	53.4	100	0	0
Pepper	73.3	13.3	13.4	86.6	6.6	6.8	66.6	0	33.4	73.3	13.3	13.4
Squash	46.6	0	53.4	100	0	0	60	13.3	26.7	46.6	13.3	40.1

Pre - = Pre-emergence damping-off.
Post - = Post-emergence damping-off.
Pre -, post -emergence damping-off and survival are expressed as percentage.

Table (11): Effect of cowpea seed treatment with the aqueous plant extracts on damping-off incidence

Extracts	Pre-emergence damping-off %				Post-emergence damping-off %				Survival %			
Chloroform	40	6.6	26.6	6.6	0	0	13.3	0	60	93.4	60.1	93.4
Ethyl acetate	46.6	33.3	66.6	13.3	0	0	6.6	6.7	53.4	66.7	26.8	80
Butanol	66.6	53.3	80	53.3	13.3	13.3	6.6	13.3	20.1	33.4	13.4	33.4

R. solani caused 100% pre-emergence damping-off on cowpea, while pepper and pea plants exhibited low level of pre-emergence damping-off (66.6% and 46.6%). Also, *S. sclerotiorum* caused 100% pre-emergence damping-off only on pea.

Effect of bean seeds treatment with organic solvent extracts of *Drechslera cynodontis* culture filtrates on the development of the damping-off disease

Results in Table (11) show that chloroform extract had the highest effect in controlling the damping-off disease by *F. solani* and *S. sclerotiorum* (93.4% control), and 60.1% and 60% control for the damping-off disease caused by *R. solani* and *F. oxysporum*. Ethyl acetate extract was the second best (80% control for *S. sclerotiorum* and 66.7% for *R. solani*). On the other hand, the least effective in this respect was butanol extract which gave only 33.4% control for *S. sclerotiorum*.

DISCUSSION

Only a limited number of natural products are used directly as active ingredients in crop protection. They must be sufficiently active against the target species, safe, and biologically selective, standardized for formulation and composition, and produced by easy and rapid processes, such as synthesis, extraction or fermentation. New bioactive metabolites have often been obtained by screening extracts from different microbes randomly chosen in the environment, as in the case of the herbicide bialaphos, (Rimando and Duke 2006) produced by soil *Streptomyces* spp.; or the fungicide strobilurin, produced by *Strobilurus tenacellus*, a wood-rotting fungus. This approach can be useful if applied to a general, and not to a focused, screening for novel bioactive metabolites, but it has a low percentage of success due to their different biological activities and the constraints to evaluate them, and the almost infinite number of organic compounds with low molecular weights that could be produced (Vurro 2007).

A more focused approach could increase the percentage of success in finding useful metabolites. In the case of searching for potential natural herbicides, for instance, the observation of symptoms on diseased plants in the field can be an effective method in choosing the promising organisms. In the case of potential insecticides or antibiotics, the screening among pathogens of insects or antagonists belonging to genera already known as toxin producers can increase the probability of success. Phytotoxins often act as virulence factors and are responsible for symptoms, such as chlorosis or necrosis. Selecting pathogens which cause those kind of symptoms can increase the probability of choosing interesting and novel toxin producers.

The use of purified pathogen toxins or culture filtrates as probes for resistance has been attempted during the last 10-15 years, based on considerable evidence suggesting a correlation between toxin tolerance and resistance to pathogens. Until now, toxins and culture filtrates have been mainly used as tools for early screening of segregating populations within classical breeding programs, or for direct in vitro selection of tolerant cells

and subsequent regeneration of putative resistant plants (Buiatti and Ingram 1991).

The fungal genus *Drechslera* contains several pathogens which show enhanced pathogenicity towards their hosts. The enhanced pathogenicity in these species is linked to toxin production, because nontoxin producing strains can be just as pathogenic as toxin producing strains if they are inoculated on toxin-sensitive host varieties in the presence of the host-selective toxin (Capio *et al.*, 2004). Toxins are usually a variety of acyl or ester derivatives of common structures with varying degrees of toxicity.

The results of our study indicate that, among the tested culture filtrates, only *D. cynodontis* culture filtrate was highly effective growth inhibitor against all tested fungi and the strongest inhibitors to spore germination at concentration of 70%. When using organic solvent extracts of *Drechslera cynodontis* culture filtrates, chloroform extract was the supreme growth inhibitor against all tested fungi and was highly effective in suppressing spore germination at concentration of 30 mg/ml. In greenhouse experiments, chloroform extract had the highest effect in controlling the damping-off disease on bean caused by *F. solani* and *S. sclerotiorum* (93.4% control).

Literature on the role of culture filtrates of some plant pathogenic fungi and organic solvent extracts of these fungi as a source of fungitoxic chemicals and their importance in controlling different plant pathogens are not found. Furthermore, most of the scientific literature on culture filtrates of fungi as natural phytotoxins focuses on phytotoxicity of the culture filtrates to some plants or weeds and relationship to disease symptomology and their toxins proposed as potential natural herbicides.

Drechslera is a well-known genus producing of numerous phytotoxic metabolites. These toxins belong to the ophiobolin family. The ophiobolins are a group of sesterterpenoids with a common basic structure. They are secondary metabolites produced by the pathogenic fungi that attack some crops. Currently, 23 biogenic analogs of ophiobolins have been identified, and molecular weights of ophiobolins are about 400 (Yun *et al.*, 1988). The ophiobolins were assigned into 12 subgroups (A to M, depending on the order of discovery). Each subgroup contains a characteristic structure in the carbon skeleton. For example, in ophiobolin M, the C-ring contains a double bond. In each subgroup the analogs are different in the stereochemistry of the substitutents on the ring system. For example, 6-epiophiobolin A contains an -H in the α-orientation at carbon 6, and in 3-anhydroophiobolin A, the -OH group is absent in carbon 3. The biological actions of ophiobolins are diverse. Basically, ophiobolins cause numerous detrimental effects when applied to plants. The growth of roots and coleoptiles of rice seedlings were inhibited at very low concentrations of ophiobolin A (Evidente *et al.*, 2006).

There are many reasons why natural products might be good sources of molecules or molecular templates for pesticides or at least lead to new targets of action (Thines *et al.*, 2006). New mechanisms of action for pesticides are highly desirable to fight the evolution of resistance in the target pests, to create or exploit unique market niches, and to cope with new

regulatory legislation. Comparatively little effort has been expended on determination of the sites of action of phytotoxins from natural sources, suggesting that intensive study of these molecules will reveal many more novel mechanisms of action (Duke *et al.*, 2000). Correlations of structure-activity are of utmost importance to the knowledge of the structural characteristics of the fungal metabolites and the determination of their active sites, or to hypothesize their chemical transformation to obtain more active, stable or selective compounds.

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المقاومة البيولوجية لبعض الأمراض النباتية باستخدام المنتجات الأيضية الثانوية لجنس *Drechslera*

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تم دراسة تأثير راشح مزارع تسعة أنواع مختلفة من جنس *Drechslera* بتركيزات (٣٠، ٥٠، ٧٠ %) علي النمو الميسليومي وإنبات جراثيم بعض الفطريات الممرضة للنبات. وقد أظهرت النتائج أن راشح مزارع النوع *Drechslera cynodontis* كان الأعلى تأثيرا فى تثبيط النمو الميسليومى وإنبات جراثيم الفطريات المختبرة حيث أدى إلى تثبيط النمو الميسليومى بنسبة ٥١,١-٨٦,٧ % وتثبيط إنبات الجراثيم بنسبة ٩٢-٩٨ %. كذلك تم استخلاص راشح مزارع هذا النوع بثلاثة مذيبات عضوية هى الكلوروفورم وخلات الإيثيل والبيوتانول. وقد أظهر مستخلص الكلوروفورم أفضل النتائج في تثبيط النمو الميسليومى (٦٦,٧-٨٨,٩%) وإنبات جراثيم الفطريات المختبرة (٨٨-٩٩%). وفى تجارب الصوب، سجل مستخلص الكلوروفورم أعلى مستوى من الكفاءة في مقاومة مرض سقوط البادرات في الفاصوليا المتسبب عن فطرى *Fusarium solani* و *Sclerotinia sclerotiorum* (٩٣,٤%). بينما قاوم مستخلص خلات الإيثيل فطر *Sclerotinia sclerotiorum* بنسبة ٨٠%.