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SEARCH FOR ANTIFUNGAL COMPOUNDS OF PLANT ORIGINS FOR BIOLOGICAL CONTROL OF PLANT DISEASES: (B) FROM METHANOLIC PLANT EXTRACTS

Dawood, K. M.; Y. M. Shabana; El. A. Fayzalla and El. A. El-Sherbiny

Plant Pathology Department, Faculty of Agriculture, Mansoura University, El-Mansoura 35516, Egypt

ABSTRACT

The antifungal activity of the methanolic extracts prepared from 11 plant species (mentioned in part A) was screened against 9 plant pathogenic fungi (*Alternaria alternata, Aspergillus flavus, Botrytis cinerea, Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, Sclerotinia sativa, Sclerotium bataticola* and *Sclerotium rolfsii*). Each plant extract was tested at five concentrations, namely 0, 10, 20, 30 and 40%. *Thymus vulgaris* extract showed the highest inhibitory effects on the culture growth of all tested fungi. This extract reduced the fungal dry weights by 42 to 96%. It had the lowest values of the minimum inhibitory concentration that inhibits 50% (MIC_{50}) and 90% (MIC_{90}) for all fungi. *A. alternata, A. flavus* and *S. bataticola* were the most persistent fungi against all methanolic plant extracts tested. Their growth was even promoted by most of the plant extracts. Among the plant extracts tested, *Nigella sativa* extract was the most effective one in inhibiting the spore germination of all tested fungi by 79 to 98%. In descending order, the extract of *T. vulgaris* was the second in terms of its capability of inhibition against fungal spore germination. In greenhouse experiments, the plant extract of *N. sativa* was the most effective one in the management of the damping-off disease caused by *F. solani* and *S. sativa* on cowpea plants giving 93% control. The extract of *Ricinus communis* gave the highest level of controlling the damping-off disease caused by *F. solani* (93% control).

Keywords: Allelopathy, biological control, plant extracts, antimicrobial activity, plant pathogenic fungi, cowpea damping-off

INTRODUCTION

Development of synthetic products to control plant diseases has become difficult because of strict requirements of their efficacy, selectivity, toxicology and general impact on the environment. Consequently, there is an increasing interest in evaluating other mechanisms of control, including the effect of plant metabolites on plant pathogens. Secondary compounds, considered as final products of plant metabolism or metabolite refuses, have important ecological functions for the plants which synthesize them. One of these functions is to protect the plants against infection by pathogens. Several authors verified that most plant extracts have antifungal properties. These properties depend on the plant organ used, fungal species tested, solvent used for extraction and compound dose and structure (Pinto *et al.*, 1998). Literature on the role of higher plants as a source of fungitoxic chemicals and their importance in controlling different plant pathogens are many and varied (Tripathi *et al.*, 1983; Rafiq *et al.*, 1984; Tewari and Dath, 1984). Many workers have reported antimicrobial activity of plant extracts

and recent studies on the subject showed the importance of natural chemicals as a possible source of non-phytotoxic, systemic and easily biodegradable alternative pesticides (Qasem and Abu-Blan, 1996). Therefore, the overall objective of the present study was to find natural fungicides as the first step towards development of a biological control system for controlling some plant diseases without poliuting the environment. The specific objectives of this study were: 1) to evaluate the antifungal activities of methanolic plant extracts derived from 11 plant species against some plant pathogenic fungi; 2) to determine the minimum inhibitory concentration (MIC) of these plant extracts against the mycelial growth and spore germination of these fungi; 3) to use some plant extracts for soaking seeds for controlling damping-off disease.

MATERIALS AND METHODS

Plant Materials

The plant species (and plant parts) used in the present investigation included *Allium cepa* (bulbs), *Allium sativum* (cloves), *Artemisia judaica* (leaves and flowers), *Carum carvi* (seeds), *Datura stramonium* (seeds), *Eruca sativa* (seeds), *Eucalyptus globulus* (fresh leaves), *Mentha viridis* (leaves), *Nigella sativa* (seeds), *Ricinus communis* (seeds) and *Thymus vulgaris* (leaves).

Preparation of plant extracts

1- From fresh plant parts:

The method of Lis-Balchin *et al.* (1998) was followed. The fresh materials were sliced into small pieces, then thoroughly washed with 2% aqueous sodium hypochlorite solution and sterile distilled water. The plant materials were then soaked in methanol at the rate of 1:1 (w/v) for 48 hours at room temperature. The mixture was blended in a blender for 5 min. The extract was filtered and the filtrate was evaporated to dryness using a rotary evaporator at 60-65°C. The residues of methanol extract were used to make serial concentrations of 0, 10, 20, 30 and 40% (w/v).

2- From dry plant parts:

Plant materials were dried in the shade at room temperature, ground using electrical mill into fine powder and extracted by soaking in methanol at a rate of 1:3 (w/v) for 48 hours. The extract was filtered through cheese cloth under a strong hand pressure and the solvent was removed in vacuum at 60-65°C to give a crude extract using a rotary evaporator. The crude extract was preserved under refrigeration until using and serial concentrations same as above were made up.

Pathogenicity tests of fungi used

Alternaria alternata, *Aspergillus flavus*, *Fusarium oxysporum*, *Fusarium solani*, *Rhizoctonia solani* and *Sclerotium bataticola* were isolated on potato dextrose agar (PDA) and Czapek agar media. The plates were incubated at 26±2°C for 7 days. Cultures of the tested fungi were purified using single spore or hyphal tip techniques. Identification of the pure

cultures was accomplished according to Barnett and Hunter (1979). Isolates were grown on PDA medium in petri dishes, then transferred to PDA slants and kept in a refrigerator at 5°C as a stock cultures. Fungal cultures of *Botrytis cinerea*, *Sclerotinia sativa* and *Sclerotium rolfsii* were obtained from the Plant Pathology Institute, Agricultural Research Center, Giza, Egypt. Pathogenicity tests of these isolates were conducted using 5 host plants, namely cowpea, pea, squash, faba bean and bean. Inocula of *F. oxysporum*, *F. solani*, *R. solani*, *S. sativa*, *S. bataticola* and *S. rolfsii* were prepared by growing each fungus in 500-ml-size bottles containing sand-cornmeal (SCM) medium (96% quartz sand + 4% cornmeal, water to 20% v/w), which incubated at 26± 2°C for 20 days. The inoculum of each fungus was mixed thoroughly with the soil at the rate of 1g inoculum / 1000g soil. Seeds of cowpea, pea, squash, faba bean and bean were soaked in 1% sodium hypochlorite solution for five minutes, then washed five times with sterile tap water and placed on sterilized tissue paper until dryness at room temperature. The surface sterilized seeds were planted in pots at a rate of 5 seeds/pot, and three pots were used for each treatment (fungus). Daily observation for seed germination and symptoms of pre-, or post-emergence damping-off were recorded during a one-month period after sowing.

Antifungal activity of plant extracts on the mycelial growth

Five concentrations of each tested plant extract (0, 10, 20, 30 and 40%) were used. These concentrations were prepared by adding 0, 2, 4, 6, and 8 ml of the extract to 20, 18, 16, 14, and 12 ml of potato dextrose broth (PDB) medium, respectively in a conical flask (100 ml). It was made sure that every solution (20 ml) contained equal amount of the medium ingredients (in the final concentration), so that any effect on the mycelial growth would be due to the extract concentration only. toFive flasks were used as replicates for each concentration. Flasks were inoculated with two discs of fungal culture (0.5 cm diameter) grown on PDA and incubated at 26±2°C until the mycelial growth covered the medium surface in the control flasks. The cultures were filtered through pre-weighed Whatman No.1 filter paper (Whatman International Ltd. Maidstone, England). Filter papers containing mycelium were washed with distilled water and dried in an oven at 80°C for 48 hours, then the dry weight was recorded. The antifungal activity of a tested extract was expressed as a percent inhibition of a fungus growth calculated according to the following equation:

$$\% \text{ Inhibition} = \frac{A - B}{A} \times 100$$

where: A= Fungal dry weight of the untreated control
B= Fungal dry weight of the treatment

Antifungal activity of plant extracts on germination of fungal spores

Five fungi namely, *A. alternata*, *A. flavus*, *B. cinerea*, *F. oxysporum* and *F. solani* were used in this investigation. They were grown on PDA (for *A. flavus*, *F. oxysporum* and *F. solani*) or potato carrot agar (PCA, for *A. alternata* and *B. cinerea*) media for 10 days at 26±2°C for producing spores. Spores were collected from plates by gently rubbing the agar surface after adding 15ml of Tween-80 solution (0.02% v/v) to the sporulated cultures in

remove mycelial fragments. Serial concentrations of the plant extract (0, 10, 20, 30 and 40%) containing fungal spores were made. Five flasks each containing 20 ml of each concentration were used as replicates. Two microscopic slides per flask were made and in each slide, the number of germinated spores was recorded. The germination count was taken after 24 hours. The result was expressed as percent germination of spores.

Determination of the minimum inhibitory concentration (MIC)

A- The MIC of plant extracts for fungal growth

The MIC versus fungal growth (the lowest concentration of an extract preventing visible growth of a tested fungus) was determined for each plant extract-fungal isolate complex.

B- The MIC of plant extracts versus germination of fungal spores

The MIC versus spore germination ratio (the lowest concentration that induced no germination of fungal spores) was determined for each plant extract-fungus complex.

Effect of cowpea seed-soaking in plant extracts on the development of the damping-off disease

Inocula of *F. oxysporum*, *F. solani*, *R. solani*, *S. sativa*, *S. bataticola* and *S. rolfsii* prepared in SCM medium as previously described. The inoculum of each fungus was mixed thoroughly with the soil in sterilized plastic pots (17 cm diameter x 20 cm height), containing 2.5kg soil at the rate of 1g inoculum/1000g soil, then watered every day for 7 days. Healthy seeds of cowpea were soaked in each plant extract at concentration of 30% for 4-5 hours. Seeds were also immersed at the same time in water as a check treatment. Seeds were then placed on sterilized tissue paper until dryness at room temperature. Five seeds were planted in each pot and three pots were used for each treatment. Pots were kept in greenhouse and data were recorded 20 days after sowing for pre-emergence damping-off and after 40 days for post-emergence damping-off.

Statistical analysis

Statistical analysis of experimental data were done using the statistical software package CoStat (1990). All comparisons were first subjected to one way ANOVA and significant differences between treatment means were determined using Duncan's multiple rang test (Duncan, 1955).

RESULTS

Pathogenicity tests of fungi used

Data in Table (1) proved that pathogenicity of fungal isolates were varied host plants used in the test.

All fungi tested caused damping-off disease on all host plants tested except *F. oxysporum*, which developed the disease only on cowpea, pea and squash. Cowpea plants were the most susceptible to fungal infection by tested fungi.

Antifungal activity of plant extracts on the mycelial growth

Data in Table (2) show that the extract of *T. vulgaris* had the highest inhibitory effect on the culture growth of all tested fungi.

Table 1. Pathogenicity of selected fungi against vegetable crop plants

Host plant	*F. oxysporum* Damping off			*F. solani* Damping off			*R. solani* Damping off			*S. sativa* Damping off			*S. bataticola* Damping off			*S. rolfsii* Damping off		
	Pre-	Post-	Survival	Pre-	Post-	Survival	Pre-	Post-	Survival	Pre-	Post-	Survival	Pre-	Post-	Survival	Pre-	Post-	Survival
Bean (*Phaseolus vulgaris*)	0	0	100	26.6	6.6	66.8	60	13.3	26.7	46.6	6.6	46.8	33.3	6.6	60.1	46.6	6.6	46.8
Cowpea (*Vigna unguiculata*	80	6.6	13.4	80	13.3	6.7	100	0	0	86.6	6.6	6.8	93.3	6.7	0	100	0	0
Pea (*Pisum sativum*)	46.6	0	53.4	40	13.3	46.7	100	0	0	20	6.6	73.4	73.3	13.3	13.4	33.3	6.6	60.1
Squash (*Cucurbila pepo*)	46.6	6.6	46.8	46.6	0	53.4	46.6	13.3	40.1	33.3	0	66.7	40	0	60	66.6	0	33.4
Faba bean (*Vicia faba*)	0	0	100	20	0	80	40	0	60	0	0	100	26.6	0	73.4	20	0	80

*Pre-, post-emergence damping-off and survival are expressed as percentages.

Table (2): Antifungal activity of methanolic plant extracts on the fungal growth of tested fungi

Plant extract	% Inhibition*								
	Alt. alternata	*Asp. flavus*	*B. cinerea*	*F. oxysporum*	*F. solani*	*R. solani*	*S. sativa*	*S. bataticola*	*S. rolfsii*
A. Cepa	12.8 b**	6.9 d	87.8 b	45.6 d	54.3 c	12.8 f	75.4 b	8.5 d	71.9 c
A. Salivum	-17.7 f	15.7 c	77.9 c	76 b	69.7 b	52.3 e	36 d	-10.1 g	63 d
Ar. Judaica	-70.7 k	-52.5 i	64.4 e	-8.3 j	24.9 f	-41.7 k	9.5 h	-28.7 h	25.5 f
C. carvi	-50 h	-34.3 g	38.7 g	18.4 g	6.3 h	-34.4 j	2.4 i	-64.9 j	0 j
D. stramonium	-52.4 i	-51.5 i	-5.9 j	18.4 g	6.3 h	-34.4 j	10 g	23.9 b	11.5 i
Er. sativa	3 c	-43.6 h	39.2 g	12.4 h	6.3 h	67.4 c	-11.4 k	11.7 c	88.5 b
Eu. globulus	-47.6 g	22.1 b	35.1 h	20.7 f	14 g	-25.7 l	10 g	11.7 c	23.4 g
M. viridis	-54.3 j	14.7 c	-5.9 j	-21.7 k	-39.8 j	-12.4 h	18.5 e	-39.4 i	-7.3 k
N. sativa	-1.2 e	6.9 d	74.3 d	27.2 e	50.7 e	72.9 b	58.3 c	-1.1 f	32.3 e
R. communis	-68.9 k	-1 f	62.2 f	52.1 c	51.6 d	66.5 d	17.5 f	-64.4 j	20.8 h
T. vulgaris	79.3 a	42.2 a	95.5 a	95.4 a	89.6 a	89.9 a	86.3 a	88.8 a	89.6 a

*% Inhibition = (fungal dry weight of the untreated control – fungal dry weight of the treatment / fungal dry weight of the untreated control) x 100, negative values (-) = % Increase in the fungal growth over the control).

** Values within a column followed by a different letter are significantly different according to Duncan's multiple range test (P = 0.05).

The extract decreased the fungal dry weights by a range of 42 (for *A. flavus*) to 96% (for *B. cinerea*). *A. alternata*, *A. flavus* and *S. bataticola* were the most persistent fungi against the allelopathic effects of all methanolic plant extracts tested and moreover their growth was promoted by most of these plant extracts.

Antifungal activity of plant extracts on germination of fungal spores

The data on suppressing spore germination by the methanol plant extracts are summarized in Table (3). Among the plant extracts tested, *N. sativa* was the supreme one in inhibiting spore germination of all tested fungi by a range from 79 (for *A. alternata*) to 98% (for *F. oxysporum*). The extract of *T. vulgaris* exhibited strong inhibition against spore germination of four fungi, which ranged from 83 (for *A. flavus*) to 92% (for *A. alternata*), while it inhibited spore germination of *F. oxysporum* by 36%. This extract was rated as the second best in terms of its capability of inhibition against fungal spore germination.

Table(3): Antifungal activity of methanolic plant extracts on spore germination of tested fungi

Plant extract	% Inhibition *				
	Alt. alternata	*Asp.s flavus*	*B. cinerea*	*F. oxysporum*	*F. solani*
A. cepa	54.9	41.6	70	41	14.8
A. sativum	59.1	64.8	80.6	87.7	29.3
Ar. Judaica	43.8	94.3	51.7	21.9	73.7
C. carvi	43.2	89.2	65.1	39.4	44.3
D. stramonium	46.6	71.6	36.1	6.9	12.4
Er. sativa	74.3	24.1	78	32.2	21.8
Eu. globulus	26.3	13.1	87.7	10	31.3
M. viridis	41	80.9	45	7.4	13
N. sativa	78.8	93.3	82.8	97.7	88.7
R. communis	86.3	29.4	94.7	57.6	28.4
T. vulgaris	91.5	82.8	89.9	35.5	88.9

*** % Inhibition = (fungal dry weight of the untreated control – fungal dry weight of the treatment / fungal dry weight of the untreated control) x 100**

Determination of the minimum inhibitory concentration "MIC"

A- The MIC of plant extracts for fungal growth:

Data in Table (4) show that MIC_{90} values of the extract of *T. vulgaris* against the culture growth of *A. alternata*, *S. bataticola* and *S. sativa* are 40, 40 and 30%, respectively, while the other plant extracts recorded higher values of $MIC_{50, 90}$. In addition, the extract of *T. vulgaris* proved to be the most effective inhibitor against the growth of *A. flavus* comparing to the other plant extracts. However, it inhibited the growth of this fungus at concentrations of 40 and >40% as MIC_{50} and MIC_{90}, respectively.

Table (4): The minimum inhibitory concentrations (MIC)[a] of methanolic plant extracts for the culture growth of the tested fungi

Plant extract	*Alt. alternata*		*Asp. flavus*		*B. cinerea*		*F. oxysporum*		*F. solani*		*R. solani*		*S. sativa*		*S. bataticola*		*S. rolfsii*	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
A. cepa	>40	>40	>40	>40	<10	>40	40	>40	<10	>40	>40	>40	<10	>40	>40	>40	<10	>40
A. sativum	>40	>40	>40	>40	<10	>40	<10	>40	<10	>40	10	>40	30	>40	>40	>40	30	>40
Ar. judaica	>40	>40	>40	>40	20	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
C. carvi	>40	>40	>40	>40	30	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
D. stramonium	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
Er. sativa	>40	>40	>40	>40	30	>40	>40	>40	>40	>40	30	>40	>40	>40	>40	>40	<10	10
Eu. globulus	>40	>40	>40	>40	<30	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
M. viridis	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40	>40
N. sativa	>40	>40	>40	>40	<10	>40	<30	>40	<30	>40	<10	>40	10	>40	>40	>40	<30	>40
R. communis	>40	>40	>40	>40	<20	>40	10	>40	<20	>40	20	>40	>40	>40	>40	>40	<40	>40
T. vulgaris	<10	40	40	>40	<10	10	<10	10	<10	10	<10	10	<10	30	<10	40	<10	10

[a] The MIC_{50} and MIC_{90} values are the minimum concentrations of a plant extract that inhibited 50 and 90% of the growth of the tested fungi, respectively.

The lowest MIC_{90} values against the culture growth of *B. cinerea*, *F. oxysporum*, *F. solani* and *R. solani* were obtained by the extract of *T. vulgaris* (10%). On the other hand, the concentration of 40% of all other extracts was not sufficient to give MIC_{90} for the culture growth of these fungi.

Table 5. The MIC of methanolic plant extracts versus spore germination of the tested fungi

Plant extract	*Alt. alternata*		*Asp. flavus*		*B. cinerea*		*F. oxysporum*		*F. solani*	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
A. cepa	20	>40	30	>40	<10	>40	30	>40	>40	>40
A. sativum	20	>40	10	>40	<10	40	<10	30	40	>40
Ar. judaica	40	>40	<10	10	30	>40	>40	>40	<10	>40
C. carvi	40	>40	<10	30	20	>40	40	>40	30	>40
D. stramonium	30	>40	10	40	40	>40	>40	>40	>40	>40
Er. sativa	10	40	>40	>40	10	>40	40	>40	>40	>40
Eu. globulus	>40	>40	>40	>40	<10	30	>40	>40	40	>40
M. viridis	40	>40	<10	40	30	>40	>40	>40	>40	>40
N. sativa	<10	>40	<10	20	<10	>40	<10	<10	<10	30
R. communis	<10	40	>40	>40	<10	10	20	>40	40	>40
T. vulgaris	<10	20	<10	40	<10	30	40	>40	<10	30

***The MIC_{50} and MIC_{90} values are the minimum concentrations of a plant extract that inhibited 50 and 90% of spore germination of the tested fungi.**

B- The MIC of plant extracts for germination of fungal spores:

Data in Table (5) show that the extract of *T. vulgaris* recorded the lowest MIC_{90} (20%) against spore germination of *A. alternata* and followed by the extract of *R. communis*. The value of MIC_{90} of *A. judaica* extract was the least value for spore germination of *A. flavus* (10%). This was followed by *N. sativa* extract. *R. communias* inhibited 90% or more of spore germination of *B. cinerea* when used at concentration of 10%. This was followed by the extracts of *T. vulgaris* and *E. globulus*. The extract of *N. sativa* caused the highest level of inhibition (90%) to spore germination of *F. oxysporum* at concentration of <10%, followed by the extract of *A. sativum*. The extracts of *N. sativa* and *T. vulgaris* had the lowest MIC_{90} values against the spore germination of *F. solani* (30%), while the other plant extracts exhibited higher values of MIC_{90}

Effect of cowpea seed-soaking in plant extracts on the development of the damping-off disease

Data in Table (6) show that different level of disease control was obtained by using different plant extracts (Fig. 1, 2, 3). Extracts of *N. sativa* and *R. communis* were the most affective among the plant extracts tested against the damping-off disease caused by *F. solani* producing 93% disease control. While the extract of *N. sativa* only caused the highest level of control to the disease by *S. sativa* (93%).

Table 6. Effect of cowpea seed treatment with the methanolic plant extracts on damping-off incidence

Plant extract	Pre-emergence damping-off %						Post-emergence damping-off %						Survival %					
	F. oxysporum	*F. solani*	*R. solani*	*S. sativa*	*S. bataticola*	*S. rolfsii*	*F. oxysporum*	*F. solani*	*R. solani*	*S. sativa*	*S. bataticola*	*S. rolfsii*	*F. oxysporum*	*F. solani*	*R. solani*	*S. sativa*	*S. bataticola*	*S. rolfsii*
A.cepa	33.3 f	33.3 e	86.6 c	26.6 g	13.3 h	66.6 e	0 c	0 c	0 d	13.3 a	0 c	6.6 b	66.7 c	66.7 c	13.4 b	60.1 c	86.7 a	26.8 d
A.sativum	33.3 f	33.3 e	86.6 c	33.3 f	66.6 c	86.6 b	0 c	0 c	0 d	0 c	0 c	0 c	66.7 c	66.7 c	13.4 b	66.7 b	33.4 d	13.4 f
Ar.judaica	80 a	80 a	80 d	60 c	66.6 c	80 c	6.6 b	0 c	20 a	6.6 b	13.3 a	6.6 b	13.4 f	20 g	0 c	33.4 e	20.1 e	13.4 f
C.carvi	46.6 d	53.3 c	100 a	60 c	53.3 e	73.3 d	13.3 a	13.3 a	0 d	6.6 b	13.3 a	13.3 a	40.1 e	33.4 e	0 c	33.4 e	33.4 d	13.4 f
D.stramonium	60 c	66.6 b	86.6 c	53.3 d	60 d	73.3 d	0 c	6.6 b	13.4 b	6.6 b	6.6 b	0 c	40 e	26.8 f	0 c	40.1 d	33.4 d	26.7 d
Er.sativa	13.3 h	40 d	93.3 b	40 e	53.3 e	80 c	6.7 b	13.3 a	6.7 c	0 c	13.3 a	0 c	80 b	53.3 d	0 c	60 c	33.3 d	20 e
Eu. globulus	40 e	80 a	100 a	86.6 a	86.6 b	80 c	0 c	0 c	0 d	6.6 b	0 c	6.6 b	60 d	20 g	0 c	6.8 g	13.4 f	13.4 f
M.viridis	73.3 b	66.6 b	100 a	66.6 b	86.6 b	86.6 b	13.3 a	13.3 a	0 d	13.3 a	0 c	0 c	13.4 f	20.1 g	0 c	20.1 f	13.4 f	13.4 f
N.sativa	13.3 h	6.6 g	86.6 c	6.6 h	40 f	66.6 e	0 c	0 c	0 d	0 c	0 c	0 c	86.7 a	93.4 a	13.4 b	93.4 a	60 c	33.4 c
R.communis	20 g	6.6 g	73.3 e	33.3 f	40 f	46.6 f	0 c	0 c	6.6 c	0 c	0 c	0 c	80 b	93.4 a	20.1 a	66.7 b	60 c	53.4 b
T.vulgaris	33.3 f	20 f	86.6 c	33.4 f	20 g	33.3 g	0 c	0 c	0 d	6.6 b	0 c	0 c	66.7 c	80 b	13.4 b	60 c	80 b	66.7 a
control	80 a	80 a	100 a	86.6 a	93.3 a	100 a	6.6 b	13.3 a	0 d	6.6 b	6.7 b	0 c	13.4 f	6.7 h	0 c	6.8 g	0 g	0 g

Values within a column followed by the same letter are not significantly different according to Duncan's multiple range test ($P = 0.05$).

Fig. (1): Effect of soaking of cowpea seeds in the methanolic extract of *Ricinus communis* on the development of the damping-off disease in soil infested with *Fusarium solani*. Left to right: check, methanolic extract alone (no pathogen), pathogen + extract, pathogen alone (no extract).

Fig. (2): Effect of soaking of cowpea seeds in the methanolic extract of *Nigella sativa* on the development of the damping-off disease in soil infested with *Sclerotinia sativa*. Left to right: check, methanolic extract alone (no pathogen), pathogen + extract, pathogen alone (no extract).

Fig. (3): Effect of soaking of cowpea seeds in the methanolic extract of *Allium cepa* on the development of the damping-off disease in soil infested with *Sclerotium bataticola*. Left to right: check, methanolic extract alone (no pathogen), pathogen + extract, pathogen alone (no extract).

DISCUSSION

The methanolic extract of *T. vulgaris* was the most effective extract in inhibiting the mycelial growth of all fungi tested, considering it had the lowest MIC values for all fungi, while it was the second best, after *N. sativa*, in suppressing spore germination. These findings are supported, in part, with many researchers. Hitoko *et al.*, (1980) found that the essential oil of thyme at 400 µg/ml completely inhibited the mycelial growth of *A. flavus*. Also, this essential oil completely inhibited conidium germination and germ tube growth of *B. cinerea* at the lowest concentration tested (1000 ppm) (Antonov *et al.*, 1995), while, it inhibited growth of this fungus at concentration of 25 ppm (Arras *et al.*, 1995). Also, Zambonelli *et al.*, (1996) found that the thyme oil inhibited fungal growth of *R. solani* and *F. solani* by 50 %. Investigations with thin-layer chromatography implicated thymol extracted from *T. vulgaris* as a major antifungal active compound (Riebau *et al.*, 1995). Thymol as the major constituent in the extract of *T. vulgaris* which belongs to phenolic alcohol, was followed by moderate concentrations of the monoterpene hydrocarbons γ-terpinene and p-cymene (Karapinar, 1990). Extract of *T. vulgaris* contains also caffeic acid and tannins, which are effective against fungi, bacteria and viruses. These compounds belong to class Terpenoid and Polyphenols, respectively (Cowan, 1999). The results of the present study similar to these found with aqueous extracts (Mohamed, 2001). Some methanolic plant extracts have fungistatic action against spore germination of some tested fungi, but these extracts exhibited low effect in reducing the dry weight of these fungi. For example, the methanolic extract of *N. sativa* highly inhibited spore germination of *F. oxysporum* (98 %), while, this extract exhibited slight inhibition on mycelial growth of this fungus (only 27%). Also, the extract of *M. viridis* strongly inhibited spore germination of *A.flavus* (81%), however, it decreased the dry weight of this fungus by only 15%. In contrary, some methanolic plant extracts were found to be highly active against mycelial growth of some fungi tested, but were slightly active against spore germination of those fungi. For example, the extract of *T.vulgaris* was the strongest inhibitor against the fungal growth of *F. oxysporum* (95%), while it inhibited spore germination of this fungus by only 36%. Other plant extracts increased the mycelial growth of the test fungi, while these extracts strongly inhibited spore germination of these fungi. For example, the methanolic extract of *R. communis* increased the fungul dry weight of *A. alternata* by 69%, but inhibited spore germination of this fungus by 86%. The varied effects by a plant extract on the distinct biological characters of a fungus were reported also by (Dubey and Dwivedi, 1991; Mohamed *et al.*, 1996). When comparing data obtained in different studies, most publications provide generalizations about whether or not a plant extract or oil possesses activity against fungi and bacteria. However, not all provide details about the extent or spectrum of this activity. Some publications also show the relative activity of plant extracts and oils by comparing results from different extracts tested against the same organisms. Although the present investigation, as many of others, revealed that plant extracts have significant effects against fungi, it is rightful to draw attention that large number of researchers reported the

antimicrobial activity of these extracts against bacteria and virus as well. For instance, Chowdhury and Saha (1985) found that the onion extract had a high effect against urd bean (*Vigna mungo*) leaf crinkle viral disease; Grainge *et al.*, (1985) reported the high antimicrobial activity of garlic cloves extract against *Xanthomonas campestris* pv. *oryza in vitro*; Hanafy and Hatem (1991) reported the antimicrobial activity of the diethyl ether extract of *N. sativa* seeds against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*; Youssef and Hammad (1994) found that the essential oil of garlic inhibited *Bacillus careus*, *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus*; Hassanein and El-Doksch (1997) found that thyme oil was highly effective against *Agrobacterium tumefaciens*, *Pseudomonas solanacearum* and *Erwinia carotovora*.

The methanolic extract of *N. sativa* reduced the fungal dry weight of *F. oxysporum* by only 27%, but this extract was the most effective in controlling the damping-off disease caused by this fungus (87% control). Other plant extracts increased the fungal dry weight of some fungi tested, while these extracts exhibited moderate effect against damping-off disease caused by these fungi. For example, the methanolic plant extracts of *E. sativa* and *R. communis* increased the fungal dry weight of *S. sativa* and *S. bataticola* by 11% and 64%, respectively, while these extracts reduced the damping-off disease caused by these fungi by 30%. These results are in agreement with these reported by Sardsud *et al.* (1992), Vimala *et al.* (1993) and Rahhal (1997) who reported that there were variability in the antimicrobial activity of other plant extracts when used *in vitro* or *in vivo*. These results suggest that compounds with high antifungal potency could be isolated from the most active extracts. This underlines the importance of screening medicinal plants for antifungal activity before the definitive loss of their habitat. We hope that these results will provide a starting point with those reported by other works for discovering new compounds with better activity than agents currently available.

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البحث عن مثبطات فطرية ذات أصول نباتية بهدف استخدامها فى المقاومة البيولوجية لأمراض النبات: (ب) من المستخلصات النباتية الميثانولية

محمد خيرت داود، ياسر محمد نور الدين شبانة، السيد عبد المجيد فيظ الله، الشربيني عبد المنعم الشربيني

قسم أمراض النبات – كلية الزراعة – جامعة المنصورة – المنصورة – مصر

درس نشاط أحد عشر مستخلصا ميثانوليا للبصل والثوم والشيح والكراوية والداتورة والجرجير والكافور والنعناع وحبة البركة والخروع والزعتر على نمو وتجرثم تسعة من الفطريات الممرضة للنبات وهى ألترناريا ألترناتا وأسبرجلس فلافس وبوترايتس سينيريا وفيوزاريوم أوكسيسبورم وفيوزاريوم سولانى وريزوكتونيا سولانى وسكليروتينيا ساتيفا وسكليروشيم باتاتيكولا وسكليروشيم رولفسياى وذلك عند تركيزات ١٠، ٢٠، ٣٠، ٤٠ %. وقد أظهرت النتائج أن مستخلص نبات الزعتر كان الأعلى تأثيرا فى تثبيط النمو الميسليومى للفطريات المختبرة، حيث أدى إلى حدوث نقص في الوزن الجاف للفطريات تراوح من ٤٢ إلى ٩٦%. وكانت فطريات ألترناريا ألترناتا وأسبرجلس فلافس وسكليروشيم باتاتيكولا مقاومة لتأثير غالبية المستخلصات النباتية. هذا في حين أظهر مستخلص نبات حبة البركة أفضل النتائج في تثبيط إنبات جراثيم جميع الفطريات المختبرة وذلك بنسب تراوحت من ٧٩ إلى ٩٨%. وقد احتل مستخلص نبات الزعتر المرتبة الثانية في تأثيره على إنبات جراثيم الفطريات المختبرة. وفى تجارب الصوب، سجل مستخلص بذور نبات حبة البركة أعلى مستوى من الكفاءة في مقاومة مرض سقوط البادرات في اللوبيا المتسبب عن فطر فيوزاريوم سولانى وسكليروتينيا ساتيفا (٩٣%). وقد أعطى مستخلص بذور نبات الخروع نفس النسبة عند مقاومة مرض سقوط البادرات فى اللوبيا المتسبب عن فطر فيوزاريوم سولانى.