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SEARCH FOR ANTIFUNGAL COMPOUNDS OF PLANT ORIGINS FOR BIOLOGICAL CONTROL OF PLANT DISEASES:
(A) FROM AQUEOUS PLANT EXTRACTS

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ABSTRACT

Aqueous plant extracts of *Allium cepa* (bulbs), *A. 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), used at concentrations of 0, 10, 20, 30 and 40%, were evaluated *in vitro* for there allelopathic effects on mycelial growth and spore germination of nine plant pathogenic fungi i. e., *Alternaria alternata, Aspergillus flavus, Botrytis cinerea, Fusarium oxysporum, Fusarium solani, Rhizoctonia solani, Sclerotinia sativa, Sclerotium bataticola* and *Sclerotium rolfsii.* The extracts enhibited the fungal growth of the tested fungi at variable degrees. *A. sativum* extract showed the greatest inhibitory effects on mycelial growth and spore germination of all tested fungi. It inhibited the spore germination by 95 to 99% and decreased the fungal dry weight by 56 to 91%. *D. stramonium* and *N. sativa* extracts induced a significant reduction in the fungal dry weights when compared to the other plant extracts. The extracts of *A. sativum* and *E. sativa* showed the highest inhibitory effect on the spore germination. A concentration of 10% of these extracts prevented the germination of 90% of the spores of these fungi. In greenhouse experiments, all tested plant extracts caused significant protection of cowpea seeds against the infection by the soil-borne fungi, whereas the extract of *D. stramonium* produced 100% control of the cowpea damping-off caused by *F. oxysporum* while 93% control of the cowpea damping-off caused by *F. solani* was obtained when *A. sativum* extract was used.

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

INTRODUCTION

Among pesticides used to protect crops, fungicides were perceived until recently as relatively safe. The 1986 report of the American National Academy of Sciences (NAS) on pesticide residues on food indicated that fungicides pose more of a carcinogenic risk than insecticides and herbicides together (Research Council, Board of Agriculture,1987).Therefore,chemical fungicides are suspect in our food chain, and demand is increasing to find safe alternatives. Additionally, resistance by pathogens to fungicides has rendered certain fungicides ineffective, creating a need for new ones with alternative modes of action. Present activities to find both natural and synthetic fungicides focus on finding compounds that are safe to human and the environment (Wilson *et. al.*, 1997). Furthermore, the use of many synthetic fungicides that have various degrees of persistence in crop

protection has now been cautioned due to their carcinogenicity, teratogenicity and other residual toxicities. Several of the synthetic fungicides are reported to cause adverse effects on treated soil ecosystems because of their non-biodegradable nature (Pandey and Dubey,1994). The search for antimicrobial agents has continued to be concentrated on lower plants, fungi and bacteria. Less research has focused on higher plants although identified plant compounds such as berberine, emetine, quinine and sanguinarine still find specialised uses. Secondary metabolites from higher plants serve as defence agents against invading microorganisms. Some of these secondary metabolites; polygodial, anethole, safrole methyleugenol and cryptolepine are active antimicrobial compounds (Fabry *et. al.*, 1998). Higher plants in the tropics are a reservoir of different secondary metabolites and provide an almost limitless source of useful chemicals with different biological properties. Several higher plants have been found to possess outstanding fungitoxicity against mycelial growth or spore germination of different phytopathogenic fungi *in vitro* (Pandey and Dubey, 1994).

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* vesicavia ssp - 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 extraction technique was carried out according to Wilson *et al.* (1997) with slight modification. Fresh plant materials were washed with tap water followed by distilled water. Then, placed in plastic bags with sterile distilled water at the rate of 1:1 (w/v), and kept in a freezer for a minimum period of 12 hours at –20°C. The plant materials were then withdrawn from the freezer and allowed to thaw at room temperature. Freezing and thawing fractured the plant cells and the plant cell fluids was then collected in the bag corner outside the tissue. The mixture was blended for 5 min., filtered through double layers of cheese cloth, centrifuged at 12000 rpm for 30 min., and sterilized by using membrane filter of pore size of 0.22 µm. The resulted extract was kept in refrigerator at 5°C until using. The crude extract was considered as 100% concentration and serial dilutions (0, 10, 20, 30 and 40%) were made by using sterile distilled water.

2- From dry plant parts

Samples of dried plant materials were ground into fine powder in a highspeed micromill. The powder of plant parts was soaked in distilled water at the rate of 1:3 (w/v) and kept for 20-24 hours at room temperature

hand pressure. The extract was centrifuged at 12000 rpm for 30 min. and sterilized by filtering through a 0.22 µm membrane filter at 25±3°C to avoid any bacterial or fungal contamination. The resulted extract was kept in a refrigerator at 5°C until using. The crude extract was considered as 100% concentration. The crude extract was diluted with sterile distilled water to prepare serial dilutions of 0, 10, 20, 30 and 40%.

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 of certain obtained isolates was conducted by 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. Five 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

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.

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 each plate. The spore suspension was filtered through cheese cloth to 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.

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* were 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 all fungal isolates that were used were pathogenic to all or some of the 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

Table (2) summarizes the effect of each plant extract on tested fungi. The aqueous extract of *A. sativum* had the significantly highest antifungal activities against all tested fungi. Its inhibition rate to the fungal growth ranged from 55.5 (on *A. flavus*) to 91.2% (on *R. solani*). Also, *D. stramonium* extract significantly decreased the fungal dry weight of all tested fungi which ranged from 34 (for *A. flavus*) to 91% (for *R. solani*). The extract of *N. sativa* did not affect the dry weight of *A. flavus*, while it reduced the dry weifgt of the other tested fungi by 43-92%.

Antifungal activity of plant extracts on germination of fungal spores

The data for the inhibition of spore germination by the aqueous plant extracts are summarized in Table (3). Among all plant extracts tested, the extract of *A. sativum* found to be the supreme extract in terms of suppressing spore germination of all tested fungi, where it inhibited spore germination of all tested fungi by 94.6 to 98.6%. The extract of *E. sativa* was the second best, which exhibited 95 to 98% inhibition of spore germination of all tested fungi. The extract of *T. vulgaris* was the third best in terms of suppressing germination of fungal spores. It strongly inhibited spore germination of all tested fungi by 82 - 94%.

Determination of the minimum inhibitory concentration "MIC"
The MIC of plant extracts for fungal growth:

In general, significant reduction in the fungal dry weight was observed as the plant extract concentration increased. *A. sativum* followed by *D. stramonium* and *N. sativa* extracts induced the highest reductions in the fungal dry weight at their lowest concentrations (Table 4).

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. batalicola* 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 (*Cucurbita 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 aqueous plant extracts on culture 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	21.2 h**	12.8 c	80.6 d	63.6 g	81.2 b	60 d	43.3 g	1.6 g	63.1 f
A. sativum	82.4 a	55.5 a	88.8 a	88 a	88.4 a	91.2 a	88 a	78.2 a	89.4 a
Ar. judaica	62.2 d	-1.2 f	68.2 e	53.3 j	63 h	44.6 e	76.4 e	1.6 g	63.5 f
C. carvi	5.2 i	-8.5 h	20.6 i	75.5 d	2.2 j	-12.7 g	18.3 i	0.5 h	87.9 b
D. stramonium	72 c	34.1 b	85.3 c	82.1 b	76.8 d	91.2 a	83.6 d	77.7 b	81.9 d
Er. sativa	-6.7 k	-6.1 g	63.5 h	67.9 f	69.6 f	-15.4 h	59.6 f	-35.8 i	62.1 g
Eu. globulus	54.4 e	-17.7 i	66.5 g	55.4 i	56.4 i	59.6 d	42.8 h	10.9 f	16.7 i
M. viridis	-36.8 l	-43.9 l	2.4 j	-34.2 l	-33.7 l	-18.8 i	-17.8 k	-43.5 j	2.8 j
N. sativa	77.7 b	3.1 d	87.6 b	76.1 c	71.3 e	91.5 a	86.5 b	43 c	86.2 c
R. communis	30.1 g	-26.2 k	85.3 c	72.8 e	78.5 c	75.4 c	76.4 e	34.7 d	66.7 e
T. vulgaris	43 f	-25.6 j	67.6 f	61.4 h	65.7 g	88.8 b	84.1 c	32.6 e	44 h

* % 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 ($P = 0.05$).

Table 3. Antifungal activity of aqueous plant extracts on spore germination of tested fungi

Plant extract	% Inhibition *				
	Alt. alternata	*Asp. flavus*	*B. cinerea*	*F. oxysporum*	*F. solani*
A. cepa	5.6	95.9	5	15.9	11
A. sativum	97.1	97.7	98.2	94.6	98.6
Ar. judaica	80.9	26.7	83.7	96.1	80.5
C. carvi	4.9	94.5	2.6	8	7.4
D. stramonium	11.3	95.8	18	59.8	29.5
Er. sativa	96.2	94.8	96	95.4	97.8
Eu. globulus	4.7	97.5	3.2	10.7	30.6
M. viridis	9.3	17.8	10.6	5.7	37.6
N. sativa	47.1	94.4	48.3	78.5	43.2
R. communis	4	97.9	16.2	51.8	55.3
T. vulgaris	93.3	91.1	94.2	91.8	82

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

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

Plant extract	*It. alternata*		*spe. 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	20	>40	<10	>40	10	>40	40	>40	>40	>40	10	>40
A. sativum	<10	40	40	>40	<10	>40	<10	40	<10	40	<10	10	<10	30	<10	>40	<10	30
Ar. judaica	<10	40	>40	>40	<10	>40	30	>40	<10	>40	30	>40	<10	>40	>40	>40	<20	40
C. carvi	>40	>40	>40	>40	30	>40	<10	>40	>40	>40	>40	>40	>40	>40	>40	>40	<10	40
D. stramonium	<10	>40	40	>40	<10	>40	<10	40	<10	>40	<10	20	<10	>40	<10	40	<10	>40
Er. sativa	>40	>40	>40	>40	<20	>40	10	>40	<10	>40	>40	>40	<20	>40	>40	>40	<20	>40
Eu. globulus	10	>40	>40	>40	<10	>40	<20	>40	20	>40	20	>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	10	>40	>40	>40	<10	40	<10	>40	10	40	<10	10	<10	30	40	>40	<10	30
R. communis	30	>40	>40	>40	<10	40	<10	>40	10	>40	<10	40	<10	40	40	>40	<20	30
T. vulgaris	<30	>40	>40	>40	<10	>40	<20	>40	<20	>40	<10	20	<10	>40	<30	>40	<30	>40

[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 MIC of plant extracts for germination of fungal spores:

Data in Table (5) show that the aqueous extracts of *A. sativum* and *E. sativa* had the highest inhibitory effect on spore germination of all tested fungi. A concentration of 10% or less of these extracts was able to prevent or suppress the germination of 90% of the spores of these fungi.

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

Data in Table (6) show that different levels of disease control were obtained by using different plant extracts (Figs. 1, 2 and 3). The most effective extract in controlling the damping-off disease caused by *F. oxysporum* on cowpea was the extract of *D. stramonium*, which completely prevented the development of such disease (i. e. 100% survival of treated plants). While onion and garlic extracts were the best ones for the control of the disease caused by *F. solani*. *R. communis* extract was the most effective one against the disease caused by *S. sativa* and *S. bataticola*. However, *R. solani* followed by *S. rolfsii* were the most persistent fungi towards the fungicidal effect of all extracts tested.

DISCUSSION

Natural plant-derived fungicides can provide a wide variety of compounds as alternatives to synthetic fungicides. They may also prove valuable as "lead structures" for the development of synthetic compounds. It helps us to explore more intensely this rich source of fungicides. Among all plant extracts tested, the aqueous plant extract of *A. sativum* (garlic) proved to be the most effective mycelial growth inhibitor for all fungi tested, in addition to suppressing spore germination of all tested fungi by 95 to 99%. Even a concentration of 10% or less of its extract was able to prevent the germination of 90% of the spores of test fungi. These results agree with many workers who reported the antifungal activity of garlic extract against one or more of these fungi (El-Shami *et al.*, 1985; Carcia and lawas, 1990; KshemKalyani *et al.*, 1990; Hammad and Youssef, 1994; Bianchi *et al.*, 1997; Karade and Sawant, 1999; Yin and Tsao, 1999). This high antifungal activity of garlic extract is possibly due to some sulfer-containing compounds as many workers reported (Yoshida *et al.*, 1987; San-Blas *et al.*, 1989; Lawson *et al.*, 1991). They reported that garlic extract contains alliin [(+)-S-allyl-$_L$-cysteine sulfoxide] as a major sulfur-containing compound. When the raw garlic clove is damaged, alliin is hydrolyzed to sulfenate, pyruvate and ammonia by alliinase. Condensation of 2 mols of sulfenate gives allicin (diallyl thiosulfinate), a major sulfur-containing intermediate, which was isolated and identified as an antifungal and antibacterial by many researchers (Naganawa *et al.*, 1996). Allicin is rapidly converted to diallyl disulfide (DADS) and other sulfides because of its instability. Garlic extract contains also an ajoene [(E, Z)-4,5,9-trithiadodeca-1,6,11-triene-9-oxide], a derivative of allicin.

Table 5. The MIC of aqueous 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	>40	>40	<10	<10	>40	>40	>40	>40	>40	>40
A. sativum	<10	<10	<10	<10	<10	<10	<10	10	<10	<10
Ar. judaica	<10	40	>40	>40	<10	40	<10	10	10	30
C. carvi	>40	>40	<10	<10	>40	>40	>40	>40	>40	>40
D. stramonium	>40	>40	<10	<10	>40	>40	10	>40	>40	>40
Er. sativa	<10	10	<10	<10	<10	10	<10	10	<10	<10
Eu. globulus	>40	>40	<10	<10	>40	>40	>40	>40	>40	>40
M. viridis	>40	>40	>40	>40	>40	>40	>40	>40	40	>40
N. sativa	30	>40	<10	10	30	>40	<10	>40	40	>40
R. communis	>40	>40	<10	<10	>40	>40	30	>40	30	>40
T. vulgaris	<10	20	<10	30	<10	20	<10	20	10	20

*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,respectively.

Table 6. Effect of cowpea seed treatment with the aqueous 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	26.6 e	6.6 h	100 a	33.3 f	86.6 b	93.3 b	0 b	0 c	0 b	0 b	0 b	6.7 a	73.4 e	93.4 a	0 b	66.7 d	13.4 f	0 d
A. salivum	20 f	6.6 h	100 a	26.7 g	40 e	86.6 c	0 b	0 c	0 b	0 b	0 b	0 b	80 d	93.4 a	0 b	73.3 c	60 b	13.4 b
Ar. judaica	80 a	80 b	100 a	73.3 b	93.3 a	86.6 c	0 b	0 c	0 b	6.6 a	0 b	0 b	20 i	20 g	0 b	20.1 i	6.7 g	13.4 b
C. carvi	46.6 d	100 a	100 a	53.3 c	93.3 a	93.3 b	0 b	0 c	0 b	0 b	0 b	0 b	53.4 f	0 i	0 b	46.7 g	6.7 g	6.7 c
D. stramonium	0 i	46.6 e	100 a	13.3 h	40 e	100 a	0 b	6.6b	0 b	0 b	0 b	0 b	100 a	46.8 e	0 b	86.7 b	60 b	0 d
Er. sativa	60 b	33.3 f	86.6 c	46.7 d	46.6 d	80 d	0 b	0 c	0 b	0 b	0 b	6.6 a	40 h	66.7 c	13.4a	53.3 f	53.4 c	13.4 b
Eu. globulus	60 b	46.6 e	93.3 b	40 e	40 e	100 a	0 b	0 c	6.7 a	0 b	6.6 a	0 b	40 h	53.4 d	0 b	60 e	53.4 c	0 d
M. viridis	53.3 c	46.6 e	86.6 c	53.3 c	40 e	80 d	0 b	0 c	0 b	0 b	6.6 a	6.6 a	46.7 g	53.4 d	13.4 a	46.7 g	53.4 c	13.4 b
N. saliva	13.3 g	73.3 c	93.3 b	46.6 d	46.6 d	86.6 c	0 b	0 c	6.7 a	6.6 a	6.6 a	6.6 a	86.7 c	26.7 f	0 b	46.8 g	46.8 d	6.8 c
R. communis	13.3 g	20 g	86.6 c	6.6 i	13.3 f	80 d	0 b	0 c	0 b	0 b	0 b	0 b	86.7 c	80 b	13.4 a	93.4 a	86.7 a	20 a
T. vulgaris	6.6 h	66.6 d	100 a	73.3 b	73.3 c	100 a	0 b	6.6b	0 b	0 b	0 b	0 b	93.4 b	26.8 f	0 b	26.7 h	26.7 e	0 d
control	80 a	80 b	100 a	86.6 a	93.3 a	100 a	6.6 a	13 a	0 b	6.6 a	6.7 a	0 b	13.4 j	6.7 h	0 b	6.8 j	0 h	0 d

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 aqueous extract of *Datura stramonium* on the development of the damping-off disease in soil infested with *Fusarium oxysporum*. Left to right: check, aqueous extract alone (no pathogen), pathogen + extract, pathogen alone (no extract).

Fig. (2): Effect of soaking of cowpea seeds in the aqueous extract of *Allium sativum* on the development of the damping-off disease in soil infested with *Fusarium solani*. Left to right: check, aqueous extract alone (no pathogen), pathogen + extract, pathogen alone (no extract).

Fig. (3): Effect of soaking of cowpea seeds in the aqueous extract of *Ricinus communis* on the development of the damping-off disease in soil infested with *Sclerotinia sativa*. Left to right: check, aqueous extract alone (no pathogen), pathogen + extract, pathogen alone (no extract).

O'gara et al., (2000) reported that ajoene had antifungal, antibacterial, antiviral and antiprotozoal activities. Generally, the evaluation of several plant extracts for their antifungal activities indicates that they can be divided into groups according to the chemical structure of their volatile oil. High fungicidal activities can be found in the phenol-containing labiaceous species as opposed to weak activities in the 1,8-cineole-containing species (Riebau *et al.*, 1995). Different activities can be found in species, characterized by the presence of other major components, like anethole or pulegone. The extracts of the species examined in the present work differed chemically as well as quantitatively, but the inhibitory effects of the extracts were mainly due to the most abundant components rather than the other associated substances. The present study revealed that the extract of *D. stramonium* was the second best after garlic against the mycelial growth of all tested fungi, where it reduced the fungal dry weight for all fungi tested by 34 to 91%, and recorded the lowest MIC for all of them. Meanwhile, the extract of *E. sativa* was the second best after garlic against spore germination for all fungi tested, and recorded the lowest MIC values in this regard. These results are in agreement with (Malhotra and Rai, 1990; El-Korashy, 1997; Achala *et al.*, 1998). In present study, authors noticed that some plant extracts had slight or no effect on the mycelial growth of some fungi, however, they highly inhibited spore germination of tested fungi. For example, the aqueous extract of *A. cepa* exhibited slight inhibition against mycelial growth of *A. flavus* (13%), however, it was highly effective against spore germination of this fungus (96% inhibition). The aqueous extract of *T. vulgaris* is another example, it caused moderate reduction in the fungal dry weight of *A. alternata* (43%), but it strongly inhibited spore germination of this fungus (93% inhibition). In contrast, some plant extracts strongly suppressed the fungal growth, but only induced low levels of inhibition to spore germination. For example, the aqueous extract of *D. stramonium* caused remarkable reduction in the fungal dry weight (85%) of *B. cinerea*, however, it inhibited spore germination of this fungus by only 18%. Another example, the aqueous extract of *A. cepa* highly reduced the dry weight of *F. solani* (81%), but it decreased spore germination of the same fungus by 11% only. Moreover, although some plant extracts increased the mycelial growth of some fungi, these extracts were highly inhibiting to spore germination of the same fungi. For example, the aqueous extract of *E. sativa* increased the fungal dry weight of *A. alternata* by 6.7%. The same extract caused 96% inhibition in spore germination of the same fungus. In general, these findings are in agreement with Dubey and Dwivedi (1991) and Mohamed *et al.* (1996) who stated that in most cases, there was no any plant extract acts against all properties of a fungus by the same rate, but it is more probably in most cases, that a plant extract affects by different rates on the different biological characters of the fungus. As a matter of course, there are difficulties with comparing published results of the antifungal activity of some plant extracts. These arise where the common name but not thebotanical name is specified and where no data are given about the chemical composition of the extract. Also, there are many different methods used to investigate the antifungal activity and the results obtained by

these methods are not always directly comparable. These reasons may, in part, explain the differences in results obtained by different research groups.

The efficacy of some plant extracts as seed protectants against infection by the soil-borne fungi was evaluated in greenhouse experiments. Tested plant extracts caused different degrees of protection of cowpea seeds against infection by the soil-borne fungi when compared with the untreated seeds. Present data indicated that *R. solani* was the most persistent fungus against the allelopathic effects of all aqueous plant extracts tested. Although some plant extracts induced low inhibition levels against the mycelial growth or spore germination of some fungi tested, these extracts caused the highest level of control to damping-off disease by these fungi. For example, the aqueous extract of *R. communis* exhibited slight inhibition against mycelial growth of *S. bataticola* (35%). However, this extract was the most effective one against the damping-off disease caused by this fungus (87% protection). Other extracts even induced the growth of some fungi tested, while exhibited a moderate effect against damping-off disease caused by these fungi. For instance, the extracts of *E. sativa* and *M. viridis* increased the fungal dry weight of *S. bataticola* by 36 and 44%, respectively, while these extracts reduced the incidence of the damping-off disease caused by this fungus by 53%. These results are in agreement with Sardsud *et al.* (1992), Vimala *et al.* (1993), Pandey and Dubey (1994) and Rahhal (1997). The present study may provide another starting point with these reported by other works for discovering new compounds with better antifungal potency than agents currently available. Also, attention should be directed to screening of medicinal plants for antifungal activity before the definitive loss of their habitat.

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

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فى محاولة للبحث عن مركبات ذات أصل نباتى يكون لها تأثير مضاد للفطريات الممرضة كبديل لاستخدام المبيدات، درس تأثير المستخلصات المائية لنباتات البصل والثوم والشيح والكراوية والداتورة والجرجير والكافور والنعناع وحبة البركة والخروع والزعتر بتركيزات (١٠، ٢٠، ٣٠، ٤٠ %) على النمو الميسليومي وإنبات جراثيم تسعة من الفطريات الممرضة للنبات وهى ألترناريا ألترناتا وأسبرجلس فلافس وبوتراياتس سينيريا وفيوزاريوم أوكسيسبورم وفيوزاريوم سولانى وريزوكتونيا سولانى وسكليروتينيا ساتيفا وسكليروشيم باتاتيكولا وسكليروشيم رولفسياى. وأظهرت الدراسة أن لمستخلص فصوص الثوم قدرة عالية من التثبيط للنمو الميسليومي وإنبات جراثيم الفطريات المختبرة حيث أدى إلى إنقاص الوزن الجاف للفطريات بنسبة ٥٦-٩١ % وتثبيط إنبات الجراثيم بنسبة ٩٥-٩٩ %. وكان لمستخلص كل من الداتورة وحبة البركة تأثيرا جيدا في تقليل النمو الميسليومي للفطريات حتى عند استخدام تركيزات منخفضة منهما مقارنة بالمستخلصات الأخرى، وقد أعطت مستخلصات الثوم والجرجير أفضل النتائج في تثبيط إنبات جراثيم الفطريات المختبرة، حيث وجد أن تركيز ٢٠ % لكل منهما قام بتثبيط إنبات ٩٠ % من جراثيم هذه الفطريات. وفى تجارب الصوب، أعطت جميع المستخلصات النباتية درجات متباينة لمقاومة مرض سقوط البادرات في اللوبيا، كما أظهرت النتائج أن لمستخلص بذور نبات الداتورة قدرة على مقاومة المرض والمتسبب عن فطر فيوزاريوم أوكسيسبورم (بنسبة ١٠٠%). بينما قاوم مستخلص الثوم فطر فيوزاريوم سولانى بنسبة ٩٣,٤% المسبب الثانى لأعراض سقوط البادرات فى اللوبيا.