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Inhibition of Phytopathogenic Fungi by Extracts from Medicinal Plants in Jordan

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Abstract: Investigation of the inhibitory effect of extracts from 4 medicinal plants were carried out against four plant pathogenic fungi: *Rhizoctonia solani*, *Fusarium oxysporum*, *Verticillium* sp. and *Penicillium* sp. The highest growth inhibition of all fungi was observed with *Varthemia iphionoides* (E4), which gave (44.8%), of inhibition for *Verticillium* sp., followed by *Rhizoctonia solani* (42.9%), *Fusarium oxysporum* (42.7%) and *Penicillium* sp. (18.2%). All extracts showed the highest inhibitory effect against the plant fungi at 1000 ppm except the extract from *Phlomis viscosa* (E2) which showed the highest inhibitory effect at 500 ppm.

Key words: Medicinal plants, phytopathogenic fungi, extracts, Jordan

INTRODUCTION

Medicinal plants have been the subject of human curiosity and need. Many places in Jordan are rich in plants with various biological and pharmaceutical activities including antifungal, antibacterial, insecticidal activities (Al-Mughrabi, 2003). In Jordan, vegetable production suffered from big losses caused by plant pathogenic fungi and this should be greatly considered.

In previous research showed *Salvia indica* gave the highest growth inhibition on five pathogenic fungi collected from the Jordan environment (Khalil *et al.*, 2005).

The essential oils extracted from various plants have been shown to have significant antifungal properties (Hoffman *et al.*, 2004; Singh *et al.*, 1980). This plant contains resin, saponin, organic acids and alkaloids (Sofowara, 1984). A number of compounds have been isolated from these medicinal plants and their biological activities such as antiviral, antitumour and antimutagenic effects have been studied (Sofowara, 1982; Yaniv *et al.*, 1987; Hoffman *et al.*, 2004). However, few studies on Jordanian medicinal plants were carried out; this is set up to determine the antimicrobial effects of some medicinal plants. The inhibitory effects of plant extracts on aflatoxin synthesis have also been examined (Bhatnagar and McCormick, 1988; Zeringue and Bhatnagar, 1990). The extract of *Azadirachta indica* was observed to be a good inhibitor for the growth of *Aspergillus flavus* and *Aspergillus parasiticus* and their *in vitro* toxin production (Bhatnagar and McCormick, 1988; Bhatnagar *et al.*, 1990; Zeringue and Bhatnagar, 1990). The oil of *Ocimum canum*

exhibited a broad range of activity against 13 fungi, including human pathogens (Bhargava *et al.*, 1981). Bashar and Rai evaluated the antifungal properties of *Clematis gouriana* against some pathogenic root infecting fungi of chickpeas. Mathela screened 12 terpenoids for activities against growth of *Aspergillus*, *Penicillium* and *Fusarium* species and of these found thymol and carvacrol to be more active than nystatin and talsutin. The use of plants or plant material as fungicide are of great importance which needs more attention (Bodde, 1982). According to the economical loss of vegetable caused by fungus and studies on the antifungal activity of medicinal plant against plant pathogens are rare in Jordan. Therefore, the importance and the aim of this study were to investigate the antifungal activity of several medicinal plants (*Achillea biebersteiri*, *Phlomis viscosa*, *Arum hygrophilum* and *Varthemia iphionoides*) collected from different locations in Jordan against the following plant pathogenic fungi (*Rhizoctonia solani*, *Fusarium oxysporum*, *Verticillium* sp. and *Penicillium* sp.).

MATERIALS AND METHODS

Plant material: Four plants collected from different areas in Jordan (between April and June of 2005) were used in this study (Table 1).

Fungal strains: The plant pathogenic fungi used in this study were collected from various locations in Jordan: *Rhizoctonia solani*, *Fusarium oxysporum*, *Verticillium* sp.

Table 1: Medicinal plants collected from Jordanian environment and used from extraction preparation

Scientific name	English name	Plant part used for extraction	Area from which plant collected
<i>Achillea biebersteini</i>	Yarrow	Aerial part	Mafraq
<i>Phlomis viscosa</i>	Jerusalem sage	Aerial part	Upper, Lower Jordan, Valley, Dead sea, Amman, Petra, salt
<i>Arum hygrophilum</i>	Green Arum, water arum	Aerial part	Azraq, um-Jamal
<i>Varthemia iphionoides</i>	Common varthemia	Aerial part	Salt, Amman, north part of Jordan

Table 2: Plant pathogenic fungi collected from various Locations in Jordan

Fungus	Host	Plant part	Location
<i>Rhizoctonia solani</i>	Cucumber	Roots	Jarash
<i>Fusarium oxysporum</i>	Lettuce	Leaves	Al-Yadoda
<i>Verticillium</i> sp.	Potato	Leaves	Al-Baqa
<i>Penicillium</i> sp.	Tomato	Leaves	Al-Humra

and *Penicillium* sp. (Table 2). All fungal isolates were identified at species or genus level and deposited in fungal collection bank at Department of Biotechnology of Al-Balqa Applied University, Al-Salt, Jordan.

Fungal isolates were maintained on potato dextrose agar (PDA, Difco Laboratories, Detroit, MI USA) and the culture were stored at room temperature and subculturing once a month. The isolates were allowed to grow for 7-10 days before they were used in the microbial studies.

Preparation of extracts: Plant material was dried in shade at room temperature and then ground by using a blender. A 250 g of powdered plant material were soaked in 1.25-1.5 L of 95% ethanol for 5 days at room temperature. The mixture was stirred daily by shaker for regular infusion. After a five-day period, the extract was filtered by using Whatman filter paper No. 1 (ALBET®). The filtrate was dried using a rotary evaporator at 60°C.

The final dried extract was stored in labeled sterile glass bottles at -20°C until used (Daouk *et al.*, 1995) from plant path.

Antifungal activity: Extracts from the four test plants were diluted in (Dimethyl Sulfoxide) DMSO (10 mL as final volume). The 10 mL of DMSO including the plant extract was added to 240 mL of (Potato Dextrose Agar) PDA to give a final concentration of 100, 250, 500 and 1000 ppm for each extract and then the resulting medium was poured in plates. Control plates received only DMSO in PDA without plant extract.

Inoculum plugs from the actively growing margin of Petri plate cultures of each fungal isolate was placed face down in the center of each Petri plate using a 10-cm-long spring-loaded plunger of 5 mm diameter. Each isolate was inoculated on 3 plates for each extract and

incubated for 7-10 days at 28°C. Control plates were run along each fungal isolate and crude extract, following the same procedure as above.

Starting two days after inoculation, radial growth was recorded daily for 7 days or until the plates were overgrown. The percentage of fungal growth inhibition = [(growth in control-growth in sample)/(growth in control)×100] where growth was measured in mm as colony diameter. The values reported for minimum inhibitory concentration were average of three readings.

RESULTS AND DISCUSSION

Extracts from 4 medicinal plants from Jordan were tested against 4 phytopathogenic fungi to determine their antifungal activity. Antifungal was measured by the MIC (Minimum Inhibition Concentration). Different concentrations of each extract were tested: 100, 250, 500 and 1000 ppm. All the fungi tested in the growth inhibition assay showed various degrees of sensitivity to the 4 plant extracts obtained (Table 3).

For *Rhizoctonia solani* 42.9% of fungal growth was inhibited with E4 at 1000 ppm, followed by E2 (33.1%) at 1000 ppm, E1 (23.0%) at 1000 ppm and E3 (11.9%) at 1000 ppm. E3 had a weak antifungal effect against *R. solanre* (Table 3).

All extracts had relatively weak effect on *Penicillium* sp. with the highest of 23.7% by E2 at 500 ppm.

Fusarium oxysporum showed 42.7% of fungal growth inhibition by E4 at 1000 ppm followed by E2 (28.9%) at 1000 ppm, E1 (22.1%) at 1000 ppm and E3 (15.2%) at 1000 ppm which is the weakest (Table 3).

Verticillium sp. showed 44.8% of fungal growth inhibition by E4 at 1000 ppm, followed by E2 (36.9%) at 1000 ppm, E1 (22.3%) at 1000 ppm and E3 (14.2%) at 1000 ppm which considered as very weak antifungal effect against *Verticillium* sp.

The significance of indigenous products for plant disease control has been investigated in other studies and encouraging results are reported (Misra and Dixit, 1976; Al-Abed *et al.*, 1993).

In previous research we reported the antifungal activity (against plant pathogenic fungi) of extracts from medicinal plants collected from the Jordan environment (Khalil *et al.*, 2005). This study extended the other plants for other medicinal plant in Jordan (against pathogenic fungi) of extracts from another group of medicinal plants from Jordan. The results presented in this study support the results from the first report which shed the light on the ability of extracts from medicinal plants to be used against different fungi that cause plant diseases, but further

Table 3: Antifungal activity of plant extracts from four medicinal plants pathogenic fungi isolated in Jordan

Plant extracts	Extract concentration (ppm)	Inhibition of fungi			
		<i>Rhizoctonia solani</i>	<i>Fusarium ozysporum</i> sp.	<i>Verticillium</i> sp.	<i>Penicillium</i> sp.
	MIC				
<i>Achillea biebersteinii</i> E1	100	7.7	5.9	6.6	0.2
	250	11.1	11.6	9.7	20.0
	500	19.1	13.5	6.2	12.4
	1000	23.0	22.1	22.3	10.0
<i>Phlomis viscosa</i> E2	100	12.7	7.5	7.2	-1.7
	250	22.2	18.9	22.7	21.4
	500	25.9	20.7	26.9	23.7
	1000	33.1	28.9	36.9	18.1
<i>Arum hygrophilum</i> E3	100	5.0	5.9	-0.4	4.4
	250	8.4	7.9	5.6	9.5
	500	9.8	10.8	6.4	10.3
	1000	11.9	15.2	14.2	12.0
<i>Varthemia iphionoides</i> E4	100	5.1	6.9	-0.2	0.8
	250	19.5	20.4	19.4	12.1
	500	26.0	30.5	24.7	12.9
	1000	42.9	42.7	44.8	18.2

studies are needed to investigate the inhibitory activities of extracts from other medicinal plants on various fungi and plant pathogenic bacteria that cause severe yield losses to different crops in Jordan. In addition, efforts should be done to identify the active compounds that cause growth inhibition and try to integrate these compounds in the control programs used to reduce yield losses caused by different plant pathogens.

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