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The Inhibitory Effect of Extracts from Jordanian Medicinal Plants Against Phytopathogenic Fungi

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Abstract: The inhibitory effect of extracts from five Jordanian medicinal plants were studied against five plant pathogenic fungi: *Crupina crupinastrum*, *Teucrium polium*, *Achillea santolina*, *Micromeria nervosa* and *Ballota philistaea*. All plants showed antifungal activity against the fungi used in this study. The inhibitory effect on activity increased by increasing the concentration (from 100-1000 ppm). The highest growth inhibition of all fungi was found with *Achillea santolina* at 1000 ppm, which gave 42.2 and 42.0% of inhibition with *Fusarium oxysporum* and *Rhizoctonia solani*, respectively and the lowest were *Micromeria nervosa* and *Ballota philistaea* which gave 3.6 and 3.5%, respectively against *penicillium* sp. Results clearly indicate that the medicinal plants were used in this study are a promising source of antifungal compounds.

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

INTRODUCTION

Herbal medicines are used as therapeutic agents (Houghton, 1996; Kondratyak and Pezzuto, 2004; Mothana and Lindquist, 2005; Zaidi and Crow Jr, 2005). Many of these plants were screened for various biological and pharmacological activities including antifungal, antibacterial, insecticidal activities (Al-Mughrabi, 2003; Ismail *et al.*, 2003; Hoffman *et al.*, 2004). Some plants are used as insecticides, molluscides and rodenticide (Even, 1992; Poswal *et al.*, 1993; Anwer *et al.*, 1992; Daoud *et al.*, 1990). The plant fungal diseases are traditionally been controlled by chemical fungicides. The development of resistant strains of pathogens against various chemical fungicides and their toxic properties make the use of these chemicals limited (Lin, 1981). The use of plants or plant material as fungicide are of great importance, which needs more attention (Bodde, 1982). Various plant products like gums, oil, resins etc. are used as fungicidal (El-Sheriff *et al.*, 1980; Asthana *et al.*, 1986; Chaturvedi *et al.*, 1987; Daoud *et al.*, 1990; Cowan, 1999; Al-Mughrabi *et al.*, 2001) The biotic-control of plant diseases may have minimum adverse effect on physiological processes of plant and less environmental hazards (Isman, 1989) as well may create a fewer health problems compared to the synthetic alternatives.

In Jordan, the limitations of vegetable production caused by plant pathogenic fungi should greatly be considered. However, Studies on the antifungal activity of Jordanian medicinal plants against plant pathogens are rare. The objective of the research work was to study the antifungal activity of some medicinal plants (*Crupina crupinastrum*, *Teucrium polium*, *Achillea santolina*, *Micromeria nervosa* and *Ballota philistaea*).

Collected from different location in Jordan against plant pathogenic (*Rizoctonia solani*, *Fusarium oxysporum*, *Verticillium* sp. and *Penicillium* sp.).

MATERIALS AND METHODS

Plant material: The plants were collected from different locations in Jordan during March-May 2004 (Table 1). The identification was confirmed by Dr. Maha Syouf from the National Center for Agricultural Research and Technology Transfer in Jordan.

Fungal strains: The plant pathogenic fungi used in this study were collected from various areas in Jordan: *Rizoctonia solani*, *Fusarium oxysporum*, *Verticillium* sp. and *Penicillium* sp. (Table 2). All fungal isolates were identified at species or genus level and deposited in fungal collection bank at Faculty of Agricultural Technology of Al-Balqa Applied University, Al-Salt, Jordan.

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Table 1: Medicinal plants collected from Jordanian environment used in this study

Scientific name	English name	Family name	Plant part used for extraction	Area from which plant collected
<i>Crupina crupinastrum</i>	Crupina	Compositae	Leaves	Irbid, Jarash, Ajloun, Al-Salt, Amman, Al-Tafila
<i>Teucrium polium</i>	Poley	Labiatae	Leaves	Karak Wadi Mujeb
<i>Achilla santolina</i>	Membranous yarrow	Compositae	Leaves	Ras-Al-Naqap, Al-Tafila, Dabha
<i>Micromeria nervosa</i>	Micromera	Labiatae	Leaves	Ajloun, Wadi Shuaib
<i>Ballota philistaea</i>	Common black horehound	Labiatae	Leaves	Aqapa, Irbid, Jarash, Al-Karak, Al-Salt, Amman

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

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

Preparation of extracts: The plants were shade dried at room temperature, chopped and ground by using a blender. A 250 g of powder plant material were soaked in 1.25-1.5 L of 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 (ALBERT[®]) and dried at 60°C under reduced pressure in rotary evaporator to obtain the crude extracts.

Antifungal activity: Extracts from the six test plants were diluted in (Dimmethyl Sulfoxide) DMSO (10 mL as final volume). The 10 mL of DMSO including the plant extract was added to 240 mL of PDA to give a final concentration of 100, 250, 500 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 with 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 = $[(\text{growth in control} - \text{growth in sample}) / (\text{growth in control}) \times 100]$ where growth was measured in mm as colony diameter (Daouk *et al.*, 1995). The values reported for minimum inhibitory concentration were average of three readings.

RESULTS AND DISCUSSION

Extract of all plants used in this study were tested against four phytopathogenic fungi to determine their antifungal activity. Different concentrations of each plant extract (100, 250, 500 and 1000 ppm) were tested against pathogenic fungi. Minimum Inhibition Concentration (MIC) was measured to determine the antifungal activity. The inhibition effect of the medicinal plant used on plant pathogenic fungi were represented in Table 3.

The highest inhibition effect of all extracts was recorded at 1000 ppm. *Crubina crupinastrum* © showed 26% of inhibition on *Rhizoctonia solani*, *Teucrium polium* (T) showed 22.4% of inhibition on *Verticillium* sp., *Achilea santolina* (A) showed 42.2% of inhibition on *Fusarium oxysporum*, *Micromeria nervosa* (M) showed 16.5% of inhibition on *Veticillium* sp. and *Ballota philistea* (B) showed 24% of inhibition on *Fusarium oxysporum*.

In comparison of the antifungal activity of plant extracts (A) had the highest activity against all fungi. (T) and (B) had a moderate activity while (T) and (M) had the lowest.

Variations in the antifungal effectiveness of different extracts against different fungi was most likely due to difference in the nature of the inhibitors materials they contained. Different compounds have been isolated and purified from different plants (Seshagirirao and Prasad, 1995; Wu *et al.*, 1995; Appendino *et al.*, 2000).

The findings of this study suggest that the medicinal plant is important source of compounds that are effective against some fungi (Table 3). This investigation could play a role in limiting the severe yield losses of different vegetables in Jordan Further studies on various Jordanian medicinal plants against other pathogenic fungi are highly recommended. As well as to identify the antimicrobial compounds. This could play an important role in controlling many plant diseases (Bhargava *et al.*, 1981).

Table 3: Antifungal activity of plant extracts from five medicinal plants against pathogenic fungi isolated from Jordan

Plant extracts	Extract concentration (ppm)	% inhibition of fungi			
		<i>Rhizoctonia solani</i>	<i>Fusarium oxysporum</i>	<i>Verticillium</i> sp.	<i>Pencilium</i> sp.
<i>Crupina crupinastrum</i> (C)	100	4.8	5.7	12.3	1.1
	250	14.3	15.0	6.4	7.0
	500	21.7	20.1	11.7	10.5
	1000	26.0	24.2	19.7	13.3
<i>Teucrium polium</i> (T)	100	8.4	7.1	7.0	0.8
	250	18.2	10.3	-1.6	10.9
	500	15.9	15.0	10.5	14.2
	1000	17.0	21.1	22.4	17.5
<i>Achillea santolina</i> (A)	100	15.8	10.4	-5.0	2.2
	250	24.1	20.8	22.3	6.8
	500	32.5	28.1	25.3	13.1
	1000	42.0	42.2	28.8	25.1
<i>Micromeria nervosa</i> (M)	100	19.0	5.3	-1.9	-2.1
	250	7.2	2.7	-1.0	2.9
	500	4.8	4.4	9.0	3.7
	1000	13.2	7.0	16.5	3.6
<i>Ballota philistaea</i> (B)	100	9.2	7.8	-3.7	2.4
	250	13.4	13.3	23.1	4.3
	500	15.3	18.2	10.6	2.7
	1000	22.5	24.0	23.7	3.5

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