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ANTIFUNGAL ACTIVITY OF METHANOLIC EXTRACTS OF SOME INDIGENOUS PLANTS AGAINST COMMON SOIL-BORNE FUNGI

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Abstract

Present study was conducted to evaluate the fungicidal property of methanolic extracts of some indigenous plants of Karachi such as *Hibiscus rosa-sinensis* (leaves), *Thespesia populnea* (leaves, stem and fruit), *Withania somnifera* (leaves and stem), *Solanum surattense* (shoot) *and Melia azedarach* (fruit) against common soil-borne phytopathogens viz., *Macrophomina phaseolina, Rhizoctonia solani* and *Fusarium oxysporum* by using food poison technique. Among the eight methanolic extracts of tested parts of plants, seven showed antifungal activity, of which *T. populnea* leaves and *S. surattense* shoots inhibited growth of all three test pathogens. Leaves of *H. rosa-sinensis* did not exhibit antifungal activity. *T. populnea* (leaves and stem), *W. somnifera* (stem) and *M. azedarach* (fruit) suppressed growth of *Rhizoctonia solani* by 100%. *T. populnea* leaves and *M. azedarach* fruit inhibited growth of *M. phaseolina* by 100% and 82%, respectively *T. populnea* leaves inhibited 99% mycelial growth of *F. oxysporum.* It is concluded that the methanolic extracts of the tested indigenous plants contain natural fungicidal compounds, which can be used for the control of common soil-borne pathogens.

Key words: Soil-borne fungi, Antifungal activity, Methanolic extracts, Indigenous plants.

Introduction

Soil contains diverse communities of microorganisms some of which are capable of damaging plants and are referred to as soil-borne plant pathogens. A detrimental interaction between a soil organism and a plant is often highly specific (Agrios, 2005). Highly specialized interactions between soil organisms and plants can kill seedlings and even adult trees (Agrios, 2005). *Macrophomina phaseolina* (Tassi) Goid is one of the most damaging soil-borne pathogens, infecting about 500 plant species in more than 100 families throughout the world (Mihail & Taylor, 1995). High variation in pathogenecity or genetic diversity or both has been reported in *M. phaseolina* that confirms the ability of the pathogen to survive and adapt to the varied environmental conditions (Mayek-Perez *et al*., 2001; Baird *et al*., 2010). *Rhizoctonia solani* Kühn is another very common soil-borne plant pathogenic fungus with a great diversity of host plants, causing seed decay, damping–off, stem canker and some other diseases (Parameter, 1970). *Fusarium* species are the most diverse and widely dispersed plant pathogenic fungi (Agrios, 2005). The genus *Fusarium* poses a multifaceted threat to global crop production (Summerell *et al*., 2001). Root rot caused by *F. oxysporum* Snyd & Hans., is one of the dominant soil-borne disease due to which losses occur in both greenhouse and field condition (Hartman & Fletcher, 1991).

The fungal plant diseases are traditionally controlled by chemical fungicides but the conventional synthetic chemicals have raised many problems like pollution, development of resistant strains of the pathogens, slow biodegradation and some of them are even carcinogenic (Brent & Hollomon, 1998). Plants are natural source of food and raw material for pharmaceutical, cosmetics and perfumery industries that prevail without causing environmental disturbance. Beside their industrial use there are reports that plants have antimicrobial activities (Hassan *et al*., 2010; Moon *et al*., 2010; Khan *et al*., 2011). Several plant families like Acanthaceae, Amaranthaceae, and Magnoliaceae are known for their antifungal properties (Neerman, 2003). Many recent studies have shown that both crude extracts and purified isolated compounds from plants can be used effectively as natural fungicides for the management of plant diseases (Jabeen & Javaid, 2010; Kanwal *et al*., 2010; Riaz *et al*., 2010).

The total number of plant chemicals may exceed 40,000 and of these 10,000 are secondary metabolites whose major role in the plant is reportedly defensive (Grayer & Harborne, 1994). There were very few work carried out on the selected plant species. Hence in the present study eight methanolic extracts of different plant parts were tested *In vitro* against most common soil-borne fungi.

Materials and Methods

Collection of test plants: Plants were selected from local flora usually on the basis of the reported presence of antimicrobial properties in accordance with the available literature. The test plants *Hibiscus rosa-sinensis* L.,

Thespesia populnea (L) Sol. ex Corr, *Withania somnifera* (L.) Dunal, *Solanum surattense* Burm. F. and *Melia azedarach* L. were collected from different localities of Karachi. Leaves, stem and fruits of these plants were separated, washed with tap water, shade-dried in screen house, and ground in an electric grinder.

Preparation of methanolic extracts: Plant extracts were obtained by extraction of specific plant parts in 95% methanol (Loew, 1997). Twenty five gram of powdered plant material of each plant part was dissolved in 100 ml methanol in sterile flask covered with cotton plug to avoid evaporation and kept undisturbed at room temperature for 24 h. Subsequently, it was filtered through Whatman No.1 filter paper and then the extract was evaporated in a water bath until 25 ml extract was left in the container. This standard methanolic extract was immediately evaluated for antifungal activity by poisoned food technique (Barreto *et al*., 2002).

Isolation of test fungi: The test fungi *M. phaseolina, R. solani* and *F. oxysporum* were isolated from infected roots of chili. The infected root samples were cut into 1.5 to 2cm long pieces, surface sterilized by 1% $Ca(OCI)_2$ for 1 min and transferred on to petri dishes containing Potato Dextrose agar (PDA) medium amended with antibiotics Penicillin (@ 100,000 unit per liter) and Streptomycin (@ 0.2g per liter) and incubated for 3-6 days at 28°C.

Antifungal assay: Antifungal activity of plant material was determined by food-poisoned technique (Schmitz, 1930). Standard extracts (5 ml) was mixed with 45 ml of sterilized PDA medium and transferred equally into two sterilized Petri plates. When media solidified, the seven day old fungal culture disk of 6 mm diameter cut through sterilized cork borer and placed in the center of each Petri dishes under aseptic conditions. Petri dishes containing PDA medium with methanol only (without plant extracts) served as control. All dishes were incubated at 28°C for 7 days and radial growth of colony was measured. Each test was replicated thrice. The results were compared with control. Inhibition % was calculated by using following formula:

$$\text{Mycelial growth inhibition (\%)} = \frac{dc - dt}{dc} \text{ X } 100$$

where dc = average mycelial growth in control, dt = average mycelial growth in treatment.

Results and Discussion

Biological control has attained importance in modern agriculture to reduce the hazards of extensive and indiscriminate use of chemicals for pest and disease control (Baker & Cook, 1979). The search for antimicrobial activity from natural sources has received much attention and efforts have been directed to identify compounds that can act as suitable antimicrobial agents to replace synthetic ones. Phytochemicals derived from plants are often effective to control growth of microorganism (Kelmanson *et al.,* 2000; Ahmad & Beg, 2001). Numerous studies have been conducted with the extracts of several plants, screening antimicrobial activity often leading to discovery of new antimicrobial compounds (Tazakou *et al*., 2001; Morris & Walker 2002; Zakaria *et al.,* 2007; Devanand & Usharani, 2008; Hassan *et al*., 2010).

It was observed that methanolic extracts of the tested plants showed varied inhibitory effects against *R. solani*, *F. oxysporum* and *M. phaseolina*. Inhibition of mycelial growth ranged from 13.64-100% (Table 1). The colony diameter of pathogens were significantly different ($p<0.001$). Likewise, effect of methanolic extracts of the test plants were also significantly different ($p<0.001$). The interaction of pathogen × plant extract was also significant ($p<0.001$) (Table 2).

The highest suppression mycelial growth of *M. phaseolina* was produced by *T. populnea* (leaves) followed by *M. azedarach* (fruit), *W. somnifera* (leaves), *T. populnea* (stem), *S. surattense* (branches), *T. populnea* (fruit) and *W. somnifera* (stem) in decending order. The mycelial growth of *F. oxysporum* was also inhibited by *T. populnea* (leaves) followed by *W. somnifera* (stem), *S. surattense* (branches), *M. azedarach* (fruit), *W. somnifera* (leaves) and *T. populnea* (fruit) in decreasing order. Similarly, the mycelial growth of *R. solani* was completely inhibited by *T. populnea* (leaves and stem), *W. somnifera* (stem) and *M. azedarach* (fruit) whereas, *T. populnea* (fruit), *S. surattense* (branches) and *W. somnifera* (leaves) produced 96.29% inhibition.

Among all methanolic extracts seven extracts showed fungicidal property. Of these, maximum effect was seen in *T. populnea* leaves and *S. surattense* which inhibited the growth of all the three test pathogens while the remaining extracts inhibited the growth of one or two test pathogens.

The results of present study confirm finding of other researchers. There are numerous reports on the control of pathogenic fungi and plant diseases by the use of plant extracts. Javaid & Rehman (2011) reported antifungal activity of different solvent extracts of *M. azedarach* leaves against *M. phaseolina*. According to Mahesh & Satish (2008) methanolic leaf extract of *W. somnifera* has antimicrobial activity against plant and human pathogens. Dabur *et al*. (2004) reported methanolic extract of *S. surattense* have antifungal activity against *Aspergillus fumigatus, A. flavus* and *A. niger*. Hemaishwarya *et al.* (2009) reported antimicrobial activities of *T. populnea* and *H. rosa-sinensis*. Boughalleb *et al*. (2005) demonstrated that compounds extracted from leaves, stem and flowers of *H. rosa-sinensis* have antifungal activity against *F. oxysporum* and *R. solani* but during the present studies, it was observed that leaves extract of *H. rosa-sinensis* have no active against these pathogens.

The present study is the first effort to evaluate the anifungal activity of methanolic extracts of the tested plants against common soil-borne phytopathogens in Karachi. It may be suggested that indigenous plants investigated here have potential antimicrobial compounds which suppress the growth of fungi and their fungicidal potential can be exploited to develop eco-friendly commercial fungicides.

Table 1. Inhibition of mycelial growth of pathogens by plant extracts as compared to control.

Name of plants	Parts used	Mycelial growth inhibition %		
		R. solani	***F. oxysproum***	***M. phaseolina***
Thespesia populnea	Leaves	100	98.63	99.60
	Stem	100	0	69.00
	Fruit	96.29	13.64	66.50
Withania somnifera	Leaves	96.29	24.96	75.99
	Stem	100	95	46.99
Hibiscus rosa	Leaves	0	0	0
Solanum surattense	Leaves	96.29	81.81	67.99
Melia azedarach	Fruit	100	49.97	81.99

Table 2. F-ratios derived from ANOVA for pathogen colony diameter and plant extracts.

Source	F-ratio	P-value	$LSD_{0.05}$
Pathogen	45.89	$.001^{***}$	3.34
Plant	182.32	$.001^{***}$	5.46
Pathogen×Plant	10.50	$.001^{***}$	-

LSD= Least significant differences at p=0.05

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