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Antifungal Activity of 4',7-Dimethoxyisoflavone Against Some Fungi

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The 4',7-dimethoxyisoflavone was isolated from the leaves of *Albizzia lebbeck* for the first time. This flavonoid showed antifungal activity against some plant pathogenic fungi tested *in vitro*, e.g., *Alternaria melongenae*, *A. brassicicola*, *A. brassicae*, *Curvularia maculans*, *C. pallescens*, *C. lunata*, *Curvularia* species, *Colletotrichum* species, *Helminthosporium penniseti* and *H. speciferum*. The sensitivity of different fungi to this chemical varied considerably. *A. brassicae* was most sensitive as complete inhibition of germination was observed in all the concentrations (100 to 1000 ppm) of the chemical. Similar effect on *H. speciferum* and *Curvularia* species was also recorded at 500 ppm, whereas *H. penniseti* did not germinate at 250 ppm. *A. melongenae* and *A. brassicicola* also did not germinate at 1000 ppm while 750 ppm was inhibitory to *C. lunata* and *C. maculans*. Germination in almost all fungi was significantly inhibited at each concentration in comparison to control except *Curvularia* sp. and *H. speciferum*. Use of 4',7-dimethoxyisoflavone to control some plant diseases under field conditions has been suggested.

KEYWORDS: Antifungal activity, 4',7-Dimethoxyisoflavone, Spore germination

Synthetic fungicides are being used successfully for the control of various fungal diseases of crop plants. This has resulted into human hazards, resistance in pathogens and environmental pollution. Recent awareness of these hazardous effects warrants the use of environmentally safer alternative methods of disease control. Some of the approaches currently pursued are biological control, genetic engineering for evolving resistant varieties and induced resistance in host by biotic and abiotic means (Lyon *et al.*, 1995). The use of bio-degradable plant products specially from medicinal plants is another aspect gaining importance in plant disease control. Plants contain a wide range of antifungal compounds (Pan *et al.*, 1985) and many of them, particularly phenolics, have been implicated in the natural resistance of several plants against pathogens. Although use of plant products under field conditions is rare, Neemazal, a product of neem (*Azadirachta indica*) and ajoene, a constituent of garlic (*Allium sativum*) have recently been used successfully against powdery mildew (*Erysiphe pisi*) of pea (*Pisum sativum*) under field conditions (Prithiviraj *et al.*, 1998; Singh *et al.*, 1995).

The antifungal activity of flavonoids has been reported earlier. Among their different classes chalcones, flavonones and dihydrochalcones have been shown to possess promising antifungal activity (Pan *et al.*, 1985; Singh *et al.*, 1988). These observations prompted us to investigate the antifungal activity of 4',7-dimethoxyisoflavone isolated from *Albizzia lebbeck*.

The test fungi were isolated on potato dextrose agar (PDA) (peeled potato 250 g, dextrose 20 g, agar 15 g, distilled water 1 liter) medium from their respective hosts (Table 1) collected from the experimental farm of the Banaras Hindu University. The cultures were purified by single spore isolation technique on PDA slants and maintained by periodic transfer on the same medium for further experiments. Seven to ten-day-old cultures were used in this experiment.

Chromatographic resolution of the methanolic extracts of the leaves of *A. lebbeck* over silica gel furnished TLC single spot semi-solid, which crystallized from MeOH as light yellow needles, m.p. 300~303°C. It did not show colour with phosphomolybdic acid and ammonia vapour indicating the absence of phenolic hydroxyl group. The ethanolic solution of the compound developed pink coloration with mg and concentrated HCl, a positive test for flavonoids. The compound exhibited molecular ion peak at m/z 282 (M^+) in its mass spectrum, which supported the molecular formula as $C_{17}H_{14}O_4$. The spectral data, viz., IR, UV ^{1}HNMR and mass spectrum were identical with the reported data of 4',7-dimethoxyisoflavone (1) (Ollis, 1966). The structure of the above compound was further supported by direct comparison with authentic sample (m.p., co-TLC and superimposable IR) (Fig. 1). This is the first report of the occurrence of this flavonoid in *A. lebbeck*.

Stock solution (1000 ppm) of 4',7-dimethoxyisoflavone was prepared by dissolving 10 mg of chemical initially with a few drops of methanol in a test tube. After the chemical was completely dissolved, approx. 10 ml of distilled water was added. The methanol was then evaporated on water bath. The required concentrations (100, 200, 250, 500, 750, and 1000 ppm) of the chemical were prepared from the stock solution by diluting with distilled water. A drop (30~40 μl) of the solution was placed on a grease-free glass slide. Fungal spores (about 200~300) were mixed

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Table 1. Effect of 4',7-dimethoxyisoflavone on spore germination of som fungi

Fungus	Host	Concentration (ppm)						
		Control	100	200	250	500	750	1000
		Percent germination						
Alternaria melongenae	*Capsicum annum*	81.90	33.64**[a]	32.81**	20.76**	20.40**	15.67**	0.00**
Alternaria brassicicola	*Brassica oleracea* var. *capitata*	91.61	46.37**	30.18**	28.88**	13.15**	9.56**	0.00**
Alternaria brassicae	*Brassica campestris*	86.85	0.00**	0.00**	0.00**	0.00**	0.00**	0.00**
Curvularia maculans	*Musa paradisiaca*	84.66	28.60**	24.60**	16.10**	1.54**	0.00**	0.00**
Curvularia pallescens	*Bambusa indica*	93.99	21.46**	18.15**	30.85**	8.97**	6.41**	1.44**
Curvularia lunata	*Sesamum indicum*	88.32	15.80**	15.30**	7.97**	6.34**	0.00**	0.00**
Curvularia species	*Imparata cylendrica*	86.32	85.12	84.64	82.16	0.00**	0.00**	0.00**
Colletotrichum species	*Arundinaria falcata*	98.83	39.00**	38.00**	21.66**	6.00**	2.00**	0.83**
Helminthosporium pennisetti	*Pennisetum typhoides*	96.00	37.73**	22.46**	0.00**	0.00**	0.00**	0.00**
Helminthosporium spiciferum	*Solanum melongena*	98.34	93.77	47.86**	36.02**	0.00**	0.00**	0.00**

[a]Values vary significantly ($P \leq 0.01$). C.D. = 23.64.

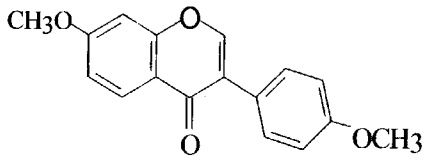

Fig. 1. Structure of 4',7-dimethoxyisoflavone (1).

in the solution with the help of a sterile inoculation needle. The slides were later placed in moist chamber made by placing two sterile moist filter papers on the lid and base of Petri plates. Such slides were then incubated at 25±2°C for 24 h for germination. The germination of the spores was observed after staining with cotton blue prepared in lactophenol under binocular light microscope (Nikon, Japan). All the experiments were conducted in triplicate.

The effect of 4',7-dimethoxyisoflavone on spore germination of some plant pathogenic fungi was seen (Table 1). The sensitivity of different fungi to this chemical varied considerably. *Alternaria brassicae* was the most sensitive as complete inhibition of germination was observed in all the concentrations (100, 200, 250, 500, 750 and 1000 ppm) of the chemical. Similar effect on *Helminthosporium speciferum* and *Curvularia* species was recorded at 500 ppm, whereas *H. penniseti* did not germinate at 250 ppm. *Alternaria melongenae* and *A. brassicicola* germination was inhibited at 1000 ppm while *Curvularia lunata* and *C. maculans* at 750 ppm showed similar effect. The germination of almost all fungal spores was significantly inhibited at each concentration in comparison to control except *Curvularia* sp. and *H. speciferum*.

The presence or absence of the pigment of spores does not seem to affect the activity of the chemical. Hyaline spores of *Colletotrichum* sp. as well as pigmented spores of *Curvularia* and *Helminthosporium* species were sensitive against this chemical. Singh *et al.* (1990) found that hyaline spores were more sensitive to ajoene as compared to pigmented ones.

Several compounds from plants are now known to possess antifungal activities (Singh *et al.*, 1999, 2000). In the present study the chemical was extracted from leaves of *A. lebbeck*. Although several flavones are already known to be antifungal but efficacy of the present compound is highy promising at a very low concentration and therefore, it is possible to envisage using this chemical as a control as a control measure against some plant diseases under field conditions.

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