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Antifungal Activity of Withametelin, a Withanolide Isolated from *Datura metel*

U. P. Singh*, Om Prakash and A. B. Ray[1]

Department of Mycology and Plant Pathology, Institute of Agricultural Sciences and
[1]Retired Professor, Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi 221005, India

Withametelin, a steroidal compound isolated from leaves of *Datura metel* L. (Solanaceae), showed antifungal activity against some plant pathogenic as well as saprophytic fungi tested *in vitro*. Except *Curvularia maculans* and *Colletotrichum* sp., spore germination of all the other 23 fungi was inhibited significantly at 125 to 1000 ppm. Out of the 25 fungi tested, *Curvularia* sp., *Cercospora abelmoschi, Heterosporium* sp., *Erysiphe cichoracearum* and *Ustilago cynodontis* were most sensitive as there was complete inhibition of germination at 1000 ppm. Similarly, spores of *Alternaria brassicae, Curvularia lunata* and *Helminthosporium pennisetti* showed less than 3% germination at the same concentration. Rest of the fungi showed more than 15% spore germination at the same concentration.

KEYWORDS: Withametelin, Antifungal activity, Spore germination

Despite widespread use of synthetic chemicals for the control of plant diseases, recent awareness about their adverse side effects prompted the use of environmentally acceptable alternative method for disease control. The approaches that are presently being persuaded are biological control, genetic engineering, use of systemic acquired resistance (SAR) with the help of biotic and abiotic agents (Lyon *et al.*, 1995) and more importantly, the use of biodegradable natural products, especially from medicinal plants (Prithiviraj and Singh, 1995). Crude as well as ethanolic extract of some plant extracts including *Datura* sp. have been tested by many workers for their efficacy against several plant pathogenic fungi *in vitro*, in glasshouses, and also under field conditions (Asthana *et al.*, 1982; Bambawole *et al.*, 1995; Chakraworthy and Paria, 1977; Chaturvedi *et al.*, 1987; Kobayashi *et al.*, 1987; Maillard *et al.*, 1987; Prithiviraj *et al.*, 1996; Reimers *et al.*, 1993; Sarma *et al.*, 1999; Singh *et al.*, 1984, 1988, 1990, 1992, 1995). Although the use of plant products under field conditions is rare and usually cost-prohibitive, neemazal®, a product of neem (*Azadirachta indica*) and ajoene, a constituent of garlic (*Allium sativum*), have recently been successfully used for the control of powdery mildew (*Erysiphe pisi*) of pea under field conditions (Prithiviraj *et al.*, 1998; Reimers *et al.*, 1993; Singh *et al.*, 1995).

Datura metel L. is a sub-glabrous shrubby herb which belongs to the family Solanaceae and grows throughout India. The dried leaves of the plant have long been known in India for their narcotic and anti-spasmodic properties (Wealth of IndiaRaw Materials, vol. III, 1952) and these activities are considered to be due to scopolamine and other tropine alkaloids present in the plant. While the presence of alkaloids in *D. metel* leaves has been known for a long time, it was rather recently that a novel steroid, withametelin, was isolated from this source as a major constituent (Sinha *et al.*, 1989). Withametelin (Fig. 1) was characterised as a member of withasteroids (Ray and Gupta, 1994), a group of steroidal lactones built on ergostane framework.

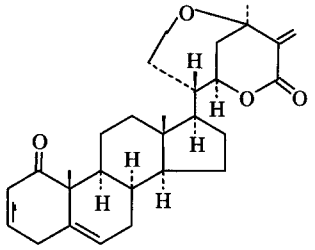

Fig. 1. Structure of withametelin.

Leaf extract of *D. metel* has been reported to exhibit plant virus inhibiting properties (Singh and Verma, 1981; Verma and Mukherjee, 1979). They have also been assayed against spore germination of *Alternaria alternata, Drechslera halodes* and *Helminthosporium speciferum* (Srivastava and Srivastava, 1998). In view of this work and the reported inhibitory action of a very common plant steroid β-sitosterol against spore germination and germ tube elongation of *Aspergillus niger* and *Botryodiplodia theobromae* (Aderiye *et al.*, 1989), it was considered worthwhile to evaluate the antifungal activity of withametelin against some phytopathogenic and saprophytic fungi. The results are presented here.

Materials and Methods

Isolation of withametelin. Withametelin (Fig. 1) was isolated from the leaves of *D. metel* following the proce-

*Corresponding author <E-mail: ups@banaras.ernet.in>

Table 1. Effect of withametelin on spore germination of some fungi

Fungus	Host	Control		Concentrations (ppm)				
		W	w + m	125	250	500	750	1000
		% Germination						
Alternaria alternata	Saprophyte	89.83	88.00	72.83**	51.67**	32.33**	29.67**	28.83**
Alternaria brassicicola	*Brassica oleracea var capitata*	91.83	91.00	88.83	84.17**	66.83**	31.17**	17.67**
Alternaria cheiranthi	*Abelmoschus esculentus*	93.67	90.00	85.33	83.00	83.00	81.00**	52.00**
Alternaria. brassicae	*Brassica juncea*	88.20	88.17	73.33**	72.50**	66.67**	2.17**	2.00**
Alternaria. melongenae	*Capsicum annum*	93.67	92.50	83.50	77.33**	69.67**	39.50**	29.50**
Alternaria. solani	*Solanum tuberosum*	92.67	91.83	80.67	75.00**	73.33**	63.67**	34.00**
Cercosporai abelmoschi	*Abelmoschus esculentus*	39.00	38.17	18.00**	3.33**	4.00**	2.00**	0.00**
Colletotrichum musae	*Musa paradisiaca*	89.50	87.92	79.33	46.00**	34.83**	31.17**	30.33**
Colletotrichum sp.	*Bombex ceiba*	90.50	88.66	85.20	84.17	83.67	82.16	81.67
Curvularia lunata	*Oryza sativa*	81.50	80.56	81.20	68.00**	41.50**	6.00**	2.00**
Curvularia maculans	*Musa paradisiaca*	91.00	90.33	85.33	84.67	82.67	81.17	80.67
Curvularia pallescens	*Bambusa* sp.	88.00	87.83	86.00	85.50	90.33	86.00	74.33**
Curvularia penniseti	*Pennisetum typhoides*	90.67	89.50	87.67	84.50	83.83	83.67	70.17**
Curvularia sp.	*Sesamum indicum*	91.17	90.67	88.50	82.17	81.50	12.00**	0.00**
Curvularia sp.	*Imperatta cylindrica*	86.83	86.20	40.00**	37.17**	31.33**	24.83**	19.50**
Erysiphe cichoracearum	*Impatiens balsamina*	24.40	23.76	20.33	19.00	9.00**	4.67**	0.00**
Fusarium sp.	*Albizzia lebbek*	78.67	78.00	78.33	66.00**	63.00**	61.00	48.67**
Fusarium udum	*Cajanus cajan*	78.68	78.17	78.67	77.00	75.33	68.67	40.20**
Helminthosporium frumentacei	*Echinochloa frumentaceum*	83.83	83.00	83.33	78.00	72.83	45.83**	16.50**
Helminthosporium pennisetti	*Pennisetum typhoides*	95.67	93.15	78.33	76.33**	55.00**	1.67**	1.33**
Helminthosporium sp.	Saprophyte	87.83	86.83	78.00	73.20**	68.20**	37.83**	37.50**
Helminthosporium spiciferam	*Solanum melongena*	90.17	89.33	88.00	86.33	86.17	86.17	74.33**
Heterosporium sp.	*Casia fistula*	89.33	88.50	50.33**	22.67**	9.50**	3.83**	0.00**
Sphaerotheca fuliginea	*Sesamum indicum*	42.17	42.00	42.00	42.00	41.67	38.33	16.67**
Ustilago cynodontis	*Cynodon dactylon*	82.83	81.67	12.17**	4.67**	4.50**	0.67**	0.00**

**Values vary significantly ($p = 0.01$) in Student's *t*-test; (w) water, (w + m) water + methanol.

dure reported by Sinha *et al.* (1989) and its purity was verified by direct comparison with authentic sample.

Antifungal bioassay. The test fungi were isolated from their respective hosts (Table 1) and maintained on slants of potato dextrose agar (PDA) (peeled potato 250 g, dextrose 20 g, agar 15 g, distilled water 1 liter) medium. The cultures were purified by single spore isolation. The spores of obligate parasites were taken directly from the infected host parts for their germination on glass slides.

Ten mg of withametelin was initially dissolved in methanol and later diluted with 10 ml sterilized distilled water. The methanol was evaporated on water bath for 5~10 min. Different concentrations (125, 250, 500, 750, and 1000 ppm) were made from the stock solution by diluting in sterilised distilled water. One drop (30~40 μl) from each concentration was placed on greasefree glass slides. Fungal spores (200~300) were picked up by a sterile needle from 7-10-day-old cultures and mixed in the solutions on glass slides. Conidia of *Erysiphe cichoracearum, Sphaerotheca fuliginea, Cercospora abelmoschi* and spores of *Ustilago cynodontis* were directly taken from the infected plant materials by lifting them with a pointed needle and mixed in the solutions of different concentrations of the chemical. Spores mixed in sterile distilled water alone and in methanolic water served as controls. The slides were then placed in moist chambers and incubated at 25±2°C for 24 h. After incubation, the spores were fixed in cotton blue prepared in lactophenol. Observations on percent germination of the fungal spores were taken under a binocular light microscope. All the experiments were conducted in triplicate. The data were subjected to Students-*t* test after angular transformation for statistical significance.

Results and Discussion

Withametelin showed significant antifungal activity against all the fungi included in the experiment at the maximum concentration (1000 ppm). However, germination of *A. alternata, A. brassicae, C. abelmoschi, Curvularia* sp., *Heterosporium* sp. and *U. cynodontis*, was significantly inhibited even at the lowest concentration (125 ppm). *C. maculans* and *Colletotrichum* sp. were least sensitive (Table 1). Spores of *Curvularia* sp., *C. abelmoschi, Heterosporium* sp., *E. cichoracearum* and *U. cynodontis* did not germinate at 1000 ppm. *A. brassicae, C. lunata* and *H. pennisetti* also showed less than 3 percent spore germination at 1000 ppm. Spores of all the fungi germinated well in both the controls (Table 1).

Steroidal compounds of plant origin are reported to be

antifungal. They affect spore germination and germ-tube elongation (Achenbach *et al.*, 1988; Aderiye *et al.*, 1989). Steroidal saponins isolated from the bulbs of *Allium ampelosperasum* exhibited antifungal activity against *Mortierella ramanniona* at 10 μg/disc concentration (Sata *et al.* 1998). Polar steroidal glycosides and steroidal glycosides from the stem bark of *Holarrhena floribundai* were effective against *Candida albicans*. These steroids exhibited antifungal activity against *Candida albicans* (Chukwurah, 1997). Several species of *Datura* are reported to contain antifungal properties in their crude extracts. Earlier studies indicate that *D. alba* and *D. stramonium* are inhibitory against several fungi (Pandey, 1982; Raghavaiah and Jayaramaiah, 1988). Aqueous leaf extract of *D. metel* has already been reported to inhibit the growth of *Pyricularia grisea* and *Helminthosporium oryzae* (Ganguly, 1994). Though previous results indicate that *Datura* contains potential antifungal compound(s) effective against a wide range of plant pathogenic as well as saprophytic fungi, the present results of antifungal activity of withametelin, a steroidal compound isolated from leaves of *D. metel* is being reported for the first time. Its efficacy at a very low concentration further indicates a possibility of its use against plant diseases under field conditions.

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