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Effect of Tetrahydropalmatine, an Alkaloid on Spore Germination of Some Fungi

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The tetrahydropalmatine alkaloid was assayed against spore germination of some saprophytic and pathogenic fungi e.g., *Alternaria solani*, *A. brassicicola*, *A. brassicae*, *A. alternata*, *Erysiphe pisi*, *Curvularia lunata*, *C. pallescens*, *C. maculans*, *Curvularia* species, *Colletotrichum* species, *C. musae*, *Helminthosporium echinoclova*, *H. pennisetti*, *H. spiciferum*, and *Heterosporium* sp. It inhibite spore germination of all the fungi tested. *Colletotrichum* spp. *Curvularia lunata*, *Helminthosporium spiciferum* and *Heterosporium* sp. were most sensitive as complete inhibition of spore germination was observed at very low concentration (200 ppm).

KEYWORDS: Antifungal activity, Spore germination, Tetrahydropalmatine

Synthetic fungicides are being used successfully for the control of various fungal diseases of crop plants. This has resulted into human health hazards, resistance in pathogen and environmental pollution. Recent awareness of these negative effects warrants the use of environmentally safe alternative methods of disease control. Some of the approaches currently pursued are: biological control, genetic engineering for evolving resistant varieties and use of induced resistance by biotic and abiotic means (Lyon *et al.*, 1995). The use of biodegradable plant products specially from medicinal plants is another aspect gaining importance in plant disease control (Prithiviraj and Singh, 1996).

Several workers have used crude plant extracts *in vitro*, in glasshouse and field conditions against several plant pathogens (Asthana *et al.*, 1987; Chakravorty and Pariya, 1977; Prithiviraj *et al.*, 1996). Various active principles isolated from the plants were proved effective against several plant pathogenic fungi *in vitro* (Kobayashi *et al.*, 1987; Maillard *et al.*, 1987, 1980; Prithiviraj *et al.*, 1997a, b; Singh *et al.*, 1988, 1990, 1992;) in glasshouse (Reimers *et al.*, 1993; Singh *et al.*, 1995) and also in the field (Prithviraj *et al.*, 1996, 1998; Sarma *et al.*, 1999). Although 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 used successfully against powdery mildew (*Erysiphe pisi*) of pea under field conditions (Prithiviraj *et al.*, 1998; Singh *et al.*, 1995).

Several alkaloids are known to affect biological functions at very low concentrations. Among such alkaloids many are known as antimicrobial (Atta-ur Rahman *et al.*, 1997; Bracker, 1994; Mahajan *et al.*, 1982; McCarthy *et al.*, 1992; Singh *et al.*, 1994, 1999, 2000; Srivastava *et al.*, 1994). However, the effect of the present compound on spore germination has not been reported so far. We report the antifungal activity of this compound for the first time.

Materials and Methods

The fungi were isolated on PDA (peeled potato 250 g, dextrose 20 g, agar 15 g, distilled water 1 liter) from their respective hosts collected from the experimental farm of the Banaras Hindu University, India.

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 10 day - old cultures were used in this experiment. The spores of obligate parasitic fungi were directly picked up from their respective hosts.

The plant *Corydalis charophylla* D.C. (Faimily: Fumariaceae) is a glabrous herb, distributed in Himalayan Region and throughout Nepal. No medicinal value and chemical compounds have earlier been reported in literature from this plant. The roots of the plant were collected from Nepal, dried and extracted with MeOH in a soxhlet extractor. The methanol extract was dried on water bath and extracted with 7% citric acid. The acidic fraction was basified with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ fraction was concentrated and chromatographed over SiO_2 gel column eluting with solvents of increasing polarity. The eluants from C_6H_6-$CHCl_3$ (1:1) on crystallization from MeOH furnished an alkaloid, as colorless granules, Rf 0.72 ($CHCl_3$-MeOH, 10:1) m.p. 139-40°C, $[\alpha]_D$-268o (c, 1.60, EtOH), $C_{21}H_{25}NO_4$ (M^+355). It exhibited absorption maximum at 254 and 287 nm like that of tetrahydroberberine. The IR, ^{1}HNMR, ^{13}CNMR and mass spectral data were identical to the reported data of 1-tetrahydropalmatine (Ruangrungsi *et al.*, 1986).

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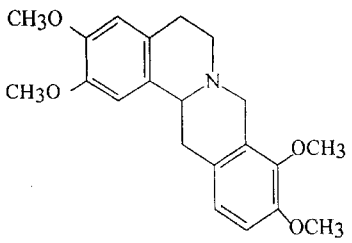

Fig. 1. Structure of *l*-Tetrahydropalmatine.

It was finally identified as 1-tetrahydropalmatine by direct comparison with authentic sample (mixed m.p., co-TLC and superimposable IR). This is the first report of the occurrence of this alkaloin in *C. chaerophylla*.

Stock solution (2000 ppm) 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. 5 ml of distilled water was added. The methanol was then evaporated on water bath. The required concentrations (200, 400, 600, 800, and 1000 ppm) of the chemical were prepared from the stock solution by diluting with distilled water. A drop (30~40 μl) of the chemical solution was placed on a grease-free glass slide. Fungal spores (about 200~300) were mixed in the solution with the help of a sterile inoculation needle. *Erysiphe pisi* conidia were directly picked up from diseased plants and mixed in the solution. The slides were later placed in moist chamber made by placing two sterile moist filter papers on the lid and base of petri plates. The spores 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 a binocular light microscope (Nikon, Japan). All the experiments were conducted in triplicate.

Results and Discussion

The effect of tetrahydropalmitine on spore germination of some plant pathogenic fungi was seen (Table 1). The sensitivity of different fungi to this chemical varied considerably. *Colletotrichum* sp., *Curvularia lunata*, *Curvularia* sp., *Helminthosporium spiciferum* and *Heterosporium* sp. were the most sensitive as complete inhibition of germination was observed in all the concentrations (200, 400, 600, 800, 1000 ppm) of the chemical. Similar effect on *C. musae* was recorded at 400 ppm, whereas *C. pallescens*, *Erysiphe pisi* and *H. echinoclova* did not germinate at 800 ppm. However, only 1000 ppm was effective against sporegrmination of *Alternaria alternata*, *A. solani* and *A. brassicicola*. The chemical was not effective at this concentration against *C. maculans* and *A. brassicae*.

The presence or absence of the pigment(s) in spores does not seem to affect the activity of the chemical. Hyaline spores of *Colletotrichum* sp. as well as pigmented *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. A number of chemical compounds isolated from plants have already been reported to be antifungal (Bracher *et al.*, 1982; Mitcher *et al.*, 1975; Prithiviraj *et al.*, 1994; Sarma *et al.*, 1999; Singh *et al.*, 1990, 1994, 1999, 2000; Srivastava *et al.*, 1994). Although several alkaloids are already known to be antifungal but the antifungal activity of the present chemical is being reported for the first time. The chemical was equally effective against biotrophic and saprophytic fungi. The

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

Fungus	Host	Concentration (ppm)					
		Percent germination					
		Control	200	400	600	800	1000
Alternaria solani	*Solanum tuberosum*	95.50[a]	89.50	89.17	87.34	58.34	0
Alternaria brassicicola	*Brassica oleracea* var. *capitata*	94.34	91.30	92.50	94.16	89.67	0
Alternaria brassicae	*Brassica campestris*	96.84	88.00	77.00	52.67	40.00**	11.67**
Alternaria alternata	Saprophyte	94.84	77.67	73.34	60.00	50.67	0
Erysiphe pisi	*Pisum sativum*	49.84	26.00	18.17	13.17	0	0
Curvularia lunata	*Oryza sativa*	98.84	0	0	0	0	0
Curvularia pallescens	*Bambusa indica*	98.67	76.17	59.00	35.00**	0	0
Curvularia maculans	*Musa paradisiaca*	98.15	91.34	89.65	84.50	81.34	12.00**
Curvularia sp.	*Imparata cylendrica*	99.00	0	0	0	0	0
Colletotrichum sp.	*Arundinaria falcata*	89.17	0	0	0	0	0
Colletotrichum musae	*Musa paradisiaca*	94.00	25.34**	0	0	0	0
Helminthosporium echinoclova	*Echinochloa crusgalli*	97.67	95.34	90.84	23.84**	0	0
Helminthosporium pennisetti	*Pennisetum typhoides*	96.34	57.34	44.84**	0	0	0
Helminthosporium spiciferum	*Solanum melongena*	97.50	1.33**	0	0	0	0
Heterosporium sp.	*Casia fistula*	90.50	0	0	0	0	0

[a]Values with double asterisk are significantly different from corresponding control values at $p \leq 0.01$ based on the Student *t*-test. C.D. at 1% = 45.95.

efficacy of the chemical is significantly high even at low concentration which indicates a possibility of its use to control plant diseases under field conditions.

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