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Effect of 1-corydalmine, an Alkaloid Isolated from *Corydalis chaerophylla* Roots on Spore Germination of Some Fungi

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1-Corydalmine, an alkaloid isolated from roots of *Corydalis chaerophylla* inhibited spore germination of some plant pathogenic as well as saprophytic fungi e.g. *Alternaria brassicae*, *A. brassicicola*, *A. solani*, *Curvularia lunata*, *C. maculans*, *C.* sp., *C. pallscens*, *Erysiphe pisi*, *Fusarium udum*, *Helminthosporium* species, *H. penniseti* and a *Heterosporium* species. 1-Corydalmine significantly inhibited spore germination of all the fungi at 100 to 1500 ppm. It was effective against all the fungi at 1500 ppm. *C. lunata* was highly sensitive to this chemical even at 250 ppm.

KEYWORDS: 1-Corydalmine, *Corydalis chaerophylla*, Spore germination

Despite widespread use of synthetic chemicals for the control of plant diseases, recent awareness about their ill effects warrants the use of environmentally acceptable alternative methods 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 biodegradable natural products especially from medicinal plants. Crude plant extracts have long been tested by many workers for their efficacy against several plant pathogenic fungi *in vitro* as well as under field conditions (Prithiviraj *et al.*, 1996). Various active principles from plants were also found effective against plant pathogens mostly *in vitro* (Sarma *et al.*, 1999; Singh *et al.*, 1990). However, some workers (Reimers *et al.*, 1993; Singh *et al.*, 1995; Prithviraj *et al.*, 1998) have found that ajoene, a constituent of garlic (*Allium sativum*), and neemazal, a product of neem (*Azadirachta indica*) are effective against spore germination of some fungi as well as in controlling powdery mildew (*Erysiphe pisi*) of pea (*Pisum sativum*) under field conditions. Nevertheless, widespread use of the plant products for disease control under field conditions is limited as they are rather cost-prohibitive and some of them are unstable.

Alkaloids are already known to affect biological functions at very low concentrations and are also known to be antimicrobial (Atta-ur-Rehman *et al.*, 1997; Bracher, 1994; Mc Carthy *et al.*, 1992; Srivastava *et al.*, 1994). 1-Corydalmine and its derivatives like (–) *cis* corydalmine-N-oxide and *trans* corydalmine-N-oxide were found to possess antiplatelet aggregation activity (Chen *et al.*, 2001). In the present study an alkaloid, 1-corydalmine was isolated from *Corydalis chaerophylla* D.C Prodr. (Family: Fumariaceae). It is a glabrous herb distributed through out Himalayas and Nepal at 2130~2770 m altitude. No chemical and biological activities have earlier been reported in literature about this plant. We studied the efficacy of 1-corydalmine against spore germination of some fungi for the first time and the results are described here.

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Materials and Methods

The plant *C. chaerophylla* was collected from Nepal. Dried and powdered roots (435 g) were extracted with methanol in a Soxhlet extractor. The methanol extract was stirred with 7% aqueous citric acid. The crude alkaloids were extracted from acidic solution after basification with NH_4OH. The crude base fraction was chromatographed over SiO_2 gel column eluting with solvents of increasing polarity. Eluants from C_6H_6-$CHCl_3$ (15 : 85) on crystallisation from methanol furnished colorless granules (30 mg), Rf. 0.62 ($CHCl_3$-MeOH, 10 : 1), m.p. 180~181°C, $C_{20}H_{23}NO_4$ (M^+341), $[\alpha]_D$–300° (c, 1.5, EtOH). It exhibited UV maxima at 283 and 253 nm like that of tetrahydroberberine. Mass spectrum exhibited molecular ion peak at m/z 341 and retro-Diel's Alder cleavage of ring C gave a base peak at m/z 190. The ^{1}HNMR and ^{13}C NMR spectral data were identical to alkaloid 1-corydalmine (Cava *et al.*, 1968). It was finally identified as 1-corydalmine by direct comparison with authentic sample (mixed m.p., co-TLC and super imposable IR (Fig. 1). This is the first report of an alkaloid in *C. chaerophylla*.

Stock solution (1000 ppm) of 1-corydalmine was dissolved in 5 mg of compound initially in a few drops of methanol and chloroform (1 : 1) in a test tube. Required concentrations of the chemical (100, 250, 500, 750, 1000 and 1500 μl^{-1}) were made by the dilution of the solution with distilled water. The chloroform and methanol were evapo-

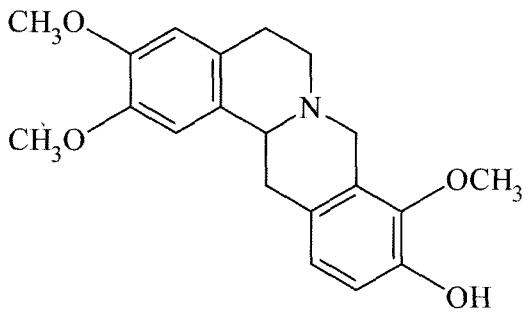

Fig. 1. Structural formula of (1)-corydalmine.

rated on water bath at 80°C. A drop (30~40 *μl*) of different concentrations of the chemical was placed separately on grease- free glass slides for studying spore germination.

The test fungi. Several fungi, e.g., *Alternaria brassicae*, *A. brassicicola*, *A. solani*, *Curvularia lunata*, *Curvularia maculance*, a *Curvularia* sp., *C. pallscens*, *Fusarium udum*, a *Helminthosporium* sp., *H. penniseti* and a *Heterosporium* sp., were isolated from their respective infected plant parts. Small portions of the infected material were incubated in moist Petri dishes for 24 h and the fungal growth developed after 24 h on potato dextrose agar (potato 250 g + dextrose + agar 20 g + distilled water 1,000 *ml*) was picked up by an inoculating sterile needle and inoculated on slants which were later purified by single spore isolation technique (Singh *et al.*, 1990). Approximately 200-300 spores of each fungus were picked up from fresh sporulating cultures by an inoculating needle aseptically and mixed in a drop of chemical kept on glass slides. The spores of *Erysiphe pisi* causing powdery mildew of pea (*Pisum sativum*) were directly removed from the infected leaves and mixed similarly with the chemical. Five slides of each fungus were prepared. All the slides were kept in Petri dishes, which were humidified by fixing moist filter paper on the lower and upper surfaces of the base and lid of the Petri dishes. All the Petri dishes were incubated at 25 ± 2°C for 24 h. After incubation a drop of cotton blue prepared in lactophenol was put on the drop of the chemical containing spores and finally covered with a cover slip. The spore germination was observed under Nikon binocular research microscope and finally the percent spore germination was calculated. The data was subjected to statistical analysis. The experiments were conducted in five triplicates.

Results and Discussion

The effect of the chemical was tested at varying concentrations starting from 100, 250, 500, 750, 1,000 to 1,500 ppm. The maximum effect of the chemical was seen on the spores of *H. penniseti* as there was no germination at 1,500 ppm and also only 21% at 1,000 ppm as compared to control (87.33%). Similarly, among the four *Curvularia* species, *C. lunata* was the most sensitive as the spore germination was significantly inhibited even at 250 ppm. Significant inhibition of spore germination in *A. brassicae* was observed at 500 ppm and above and other two species of *Alternaria* showed sensitivity at 750 ppm and above concentrations. While 250 ppm and above doses were significantly inhibitory for the germination of spores of *Heterosporium* species, similar significant effect on *Erysiphe pisi* and *F. udum* was seen at 1,000 and 1,500 ppm concentrations (Table. 1).

A number of chemical compounds isolated from plants are antifungal (Singh *et al.*, 1990; Srivastava *et al.*, 1994; Sarma *et al.*, 1999; Singh *et al.*, 1999; Basha *et al.*, 2002; Maurya *et al.*, 2001, 2002; Goel *et al.*, 2002; Sangita *et al.*, 2005). The fungi included in the present study belong to two different groups. But based on the activity of the

Table 1. Effect of 1-corydalmine on spore germination of some fungi

Fungus	Host	Concentration (ppm)							
		Control	C+M	100	250	500	750	1000	1500
		Percent germination							
Alternaria brassicae	*Brassica campestris*	86.66	85	78.5	71.83	54.83**	53.33**	34.5**	0.88**
Alternaria brassicicola	*Brassica oleracea var. capitata*	90	90	85	73	65	52.88**	42.22**	33.11**
Altenaria solani	*Solanum tuberosum*	85.66	84.52	81.16	70.5	64.16	57.66	54.33**	41.83**
Curvularia lunata	*Oryza sativa*	93.11	92.93	83.11	59.77**	45.33**	24.88**	17.33**	1**
Curvularia maculans	*Musa seprientum*	96.22	95.98	90.1	83	79	67	50	45.62**
Curvularia pallescens	*Bambusa indica*	85.83	85.1	74.33	68.16	65.66	64.5	51.88**	21.5**
Curvularia species	*Sesamum indicum*	82.83	8.089	76.66	66.33	61	59**	48**	29**
Erysiphe pisi	*Pisum sativum*	50	48	32	28	23	16	8**	3**
Fusarium udum	*Cajanus cajan*	89	88.79	73	60	51	40	17.33**	0**
Helminthosporium penniseti	*Pennisetum typhoides*	87.33	86.59	73.2	68	49	33**	21**	0**
Helminthosporium species	*Echinocloa* species	88	88.2	86	74.33	51**	30**	24.66**	2.16**
Heterosporium species	*Casia fistula*	94.88	94.54	87.77	60**	55.55**	47.55**	41.77**	8**

C = Distilled water, C + M = Distill water + few drops of methanol.
Values suffixed with double asterisks are significantly different from corresponding control values at $P \leq 0.01$ based on the student *t*-test.

chemical, it is at present difficult to conclude as to which group of fungi will be most susceptible to this chemical as the number of fungi from different groups is very low. Hence, only further detailed study on several members of other groups will decide its limit and specificity of efficacy. Nevertheless, as antifungal activity of the present compound has not been reported so far and the efficacy is significantly high at low concentrations, there is enough possibility of using this compound in plant disease control under field conditions.

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