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Inhibitory Effect of Two Alkaloids, (–)-Corydalmine and (–)-Isocorypalmine Isolated from *Corydalis chaerophylla* on Several Phytopathogenic Fungi

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Medicinal plants play important roles in controlling plant diseases as one of the safest and ecofriendly methods. These plants have been used in the form of crude extracts as well as active principles *in vitro* and under field conditions to control plant diseases. Among the active principles, alkaloids have shown significant antifungal activity. We have investigated the effect of two alkaloids viz., (–)-corydalmine and (–)-isocorypalmine isolated from *Corydalis chaerophylla*, against spore germination of some plant pathogenic and saprophytic fungal spores. Significant inhibition of spore germination at 100 μg/ml was seen against *Curvularia penniseti*, *Curvularia* sp. and *Colletotrichum gloeosporioides* by (–)-corydalmine but (–)-isocorypalmine was also effective against fungi included in the experiment.

KEYWORDS: Alkaloids, *Corydalis chaerophylla*, (–)-Corydalmine, (–)-Isocorypalmine, Spore germination

Due to extravagant use of chemical fungicides for plant disease control, health hazards on humans and plants have been witnessed in a different forms. The residual effects of chemical fungicides are long lasting as they accumulate in human and animal bodies through polluted food chains, disturbing the biodiversity and ecological balance in nature. Although the use of chemical fungicides has given a good effect in controlling plant diseases, their improper use has also developed resistance in fungal pathogens (Lyon *et al.*, 1995) besides several other adverse effects. The awareness of these wrong effects has entailed the need for searching alternatives such as biofungicides which are environmentally safe and also effective against plant diseases. Recent development of bioagents for the control of foliar, soil-borne and post-harvest diseases, has generated the socio-economic concerns on the use of chemical fungicides. Medicinal plants are one of the best alternatives of chemical fungicides as they have several active principles which have been reported by several workers to have significant effect against several plant pathogenic and saprophytic fungi (Maurya *et al.*, 2001, 2002; Amir Basha *et al.*, 2002; Lyon *et al.*, 1995; Singh and Prithiviraj, 1996). The use of crude plant extracts *in vitro* against several plant pathogenic fungi is reported by several workers (Chakravorty and Pariya, 1977; Asthana, *et al.*, 1982; Prithiviraj *et al.*, 1996). On the other hand, some others have recently used various active principles against fungal spore germination *in vitro* (Maillard *et al.*, 1987, 1989; Kobayashi *et al.*, 1987; Singh *et al.*, 1990; Prithiviraj *et al.*, 1997a, b) as well as under field conditions against some plant diseases (Reimers *et al.*, 1993; Singh *et al.*, 1995).

Alkaloids are known to be antifungal (Atta-ur-Rahman *et al.*, 1994; Maurya *et al.*, 2001, 2002). They inhibit spore germination at very low concentrations. In this report we present the efficacy of (–)-isocorypalmine and (–)-corydalmine isolated from *Corydalis chaerophylla* against spore germination of some phytopathogenic and saprophytic fungi.

Materials and Methods

Isolation of fungi. The fungi were isolated on PDA (peeled potato 250 g, dextrose 20 g, agar 15 g, distilled water 1 l.) from the infected parts of their respective host collected from the experimental farm of the Banaras Hindu University. The isolates were purified by single spore isolation technique and maintained on PDA by periodic transfer for further experiments. Isolates of all the pathogens tested were used in the experiments after 7~10 days after incubation. The conidia of *Erysiphe* sp. were collected from the infected leaves of balsam (*Impatiens balsamiana*) from the garden and used directly in the experiments.

Extraction and purification of the alkaloids. The dried roots of *Corydalis chaerophylla* (1 kg) were extracted in a soxhlet extractor with methanol. The semi-solid methanol extract (110 g) was stirred with 7% aqueous citric acid mechanically several times till it gave negative test for alkaloids. The combined acidic solution was basified with ammonium hydroxide (NH_4OH) and extracted with chloroform ($CHCl_3$) four times in a separating funnel. The total $CHCl_3$ extract was distilled on water bath

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and semi-solid of crude base (21 g) was obtained. The crude base was chromatographed over silica gel (60~120 mesh) column eluting with solvents of increasing polarity and around 55~60 *ml* eluants were collected in each flask. The entire eluted fractions were monitored by thin layer chromatography for their homogeneity. The eluants collected from benzene-chloroform (1 : 9) were mixed together and crystallized repeatedly from methanol which furnished colourless shining granules (45 mg) of compound A, m.p. 239~41°C, $[\alpha]_D^{20}$ − 296° (c, 0.50, $CHCl_3$). The eluants collected from chloroform on repeated crystallization gave colourless granules (30 mg) of compound B, m.p. 172~73°C $[\alpha]_D^{20}$ − 300° (c, 1.40, MeOH). Both the compounds, A and B, gave light orange colour with Dragendroffs reagent indicating them to be alkaloids. Both compounds were found to be phenolic in nature when tested with phosphomolybdic acid and ammonia vapour.

The molecular formula of compound A was determined as $C_{20}H_{23}NO_4$ from the molecular ion peak at m/z 341 (M^+ , base peak). It exhibited UV λ max (MeOH): 205, 230 sh and 283 nm and IR υ max. (KBr) : 2900~3500 cm^{-1} (br, OH). 500 MHz ^{1}HNMR ($CDCl_3$ + 20% MeOD) exhibited signals at δ 2.62~3.58 for three methylene protons and one proton each for C-8 and C-14. One proton doublet at δ 4.22 having J = 16 Hz is assigned for another C-8 hydrogen. Two isolated aromatic protons at δ 6.79 (C-1-H) and δ 6.61 (C-4-H) as singlets, two vicinal aromatic hydrogens as doublets (J = 8 Hz, each) at δ 6.82 (C-11-H) and δ 6.90 (C-12-H) together with three aromatic methoxyl signals at δ 3.85, 3.86 and 3.87 as singlets. The chemical shift of all the carbon signals in the 500 MHz ^{13}CNMR ($CDCl_3$ + 20% MeOD) tallies with all carbons of isocorypalmine. The mass spectrum exhibited molecular ion peak at m/z 341 and significant ion peaks at m/z 178 and m/z 164 due to retro-Diel's Alder cleavage. Other mass peaks were also in favour of the structure of isocorypalmine. The above data were identical with the reported data of (−)-isocorypalmine. It was finally characterized with authentic sample (mixed m.p., co-TLC and superimposable 1R as (−)-isocorypalmine (1) (Preininger *et al.*, 1978) (Fig. 1).

The molecular formula of compound B was determined as $C_{20}H_{23}NO_4$ (M^+ 341, base peak). It exhibited UV λ max (MeOH) : 206, 230 sh and 284 nm and IR υ max. (KBr): 3000~3500 cm^{-1} (br, OH). 500 MHz ^{1}HNMR ($CDCl_3$ + 10% CD_3OD) exhibited signals for three aromatic methoxyl groups at δ 3.72, 3.73, 3.74 and eight hydrogens in region between δ 2.40~3.42, one proton doublets at δ 4.10 (J = 16 Hz) for one proton of C-8-H, two aromatic orthohydrogens as a pair of doublet at δ 6.69 and 6.77 and two isolated aromatic hydrogens as singlet at δ 6.48 and δ 6.66, like that of corydalmine. The chemical shift of all the carbon signals in the 500 MHz

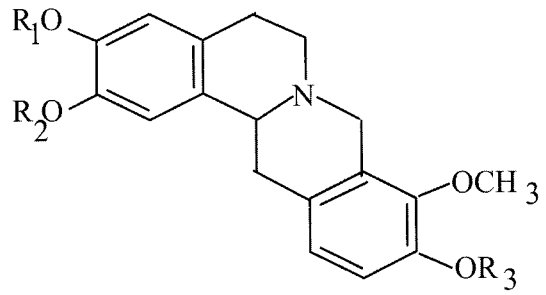

Fig. 1. Structural formula of tested plant alkaloids. (−)-Isocorypalmine (1) : $R_1 = R_3 = CH_3$, R_2 = H. (−)-Corydalmine (2) : $R_1 = R_2 = OCH_3$, R_3 = H.

^{13}CNMR ($CDCl_3$ + 10%CD_3OD) tallies with all carbons of corydalmine. The mass spectrum exhibited molecular ion peaks at m/z 341 and significant ion peaks at m/z 192 and m/z 150 due to retro-Diel's Alder cleavage. Other mass peaks were also in favour of the structure of corydalmine. The above data were identical with the reported data of (−)-corydalmine. It was finally characterized with authentic sample (mixed m. p., co-TLC and superimposable 1R) as (−)-corydalmine (Preininger *et al.*, 1978) (Fig. 1).

Fungal spore germination inhibition assay. Stock solution (2000 $\mu g/ml$) was prepared by dissolving 10 mg of each plant alkaloid initially in 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 adjusted at 80°C. The required concentrations of both the chemicals were prepared from the stock solution by diluting with distilled water separately. A drop (30~40 μl) of the chemical solution was placed on grease-free glass slides. Fungal spores (about 200~300) were mixed in the solution with the help of a sterile inoculation needle. Conidia of *Erysiphe* sp. were placed directly from the infected plant parts. The slides were later placed in moist chamber made by placing two sterile moist filter papers one on the lower surface of the lid and the other one on the base of petri plates. The petri plates containing spores were then incubated at 25 ± 2°C. 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. Data were analyzed by student t test.

Results and Discussion

The effect of (−)-isocorypalmine on spore germination of some fungi is shown in Table 1. Spore germination of six fungi was completely inhibited at 800 $\mu g/ml$ except that of *A. solani* and *Alternaria* sp.. The germination percent was extremely low in both the cases (3%) at this concentra-

Table 1. Effect of (–)-isocorypalmine on spore germination of some fungi

Fungus	Host	Percent spore germination (%) Concentration (μg/ml)							CD
		±	±-1	100	200	400	600	800	
Alternaria brassicae	*Brassica campestris*	83.00	82.00	76.00**	73.00**	3.00**	0.00	0.00	3.504
Alternaria solani	*Solanum tuberosum*	86.00	86.00	87.00	76.00**	9.00**	6.00**	3.00**	3.214
Alternaria sp.	*Capsicum annum*	79.00	78.00	80.00	21.00**	11.0**	6.00**	3.00**	8.566
Curvularia maculans	*Musa paradisica*	96.00	98.00	5.00**	2.00**	3.00**	1.00**	0.00	2.447
Curvularia penniseti	*Pennisetum typhoides*	88.00	87.00	15.00**	3.00**	0.00	0.00	0.00	5.499
Curvularia sp.	*Imperata cylendrica*	87.00	84.00	15.00**	1.00**	0.00	0.00	0.00	4.686
Erysiphe sp.	*Impatiens balsamiana*	22.00	15.00	4.00**	2.00**	0.00	0.00	0.00	3.669
Helminthosporium penniseti	*Pennisetum typhoides*	94.00	96.00	61.00**	22.00**	5.00**	2.00**	0.00	7.027

**Shows significantly different at 1% level ($p \leq 0.01$).
± Control (water), ±-1 Control + MeOH, CD = Critical Deference.

tion. Almost similar effect was seen at 600 μg/ml in other fungi also. The germination of spores of *C. penniseti* and *Curvularia* sp. and *Erysiphe* sp. was completely inhibited at 400 μg/ml while maximum germination (11.33%) was observed in *Alternaria* sp. This chemical was significantly effective in inhibiting spore germination of most of the fungi used in the present experiment. Among all the tested fungi, *A. solani*, *A. brassicae*, *Alternaria* sp. and *Helminthosporium penniseti* were less sensitive at the minimum concentration (100 μg/ml) but in other fungi spore germination was significantly inhibited (Table 1).

(–)-Corydalmine was effective in inhibiting spore germination of most of the fungi tested (Table 2). *Curvularia* species were highly sensitive against (–)-corydalmine at a very low concentration (100 μg/ml). Spore germination of *C. gloeosporioides* and *C. maculans* was completely inhibited (200 μg/ml) while in other fungi very little germination was observed. *H. penniseti* and *A. brassicae* were less affected at this concentration. Also very little spore germination was observed in *C. pennieti* (3%), *Curvularia* sp. (19%), *C. maculans* (58%) at 100 μg/ml except in *A. solani*, *A. brassicae* and *H. penniseti* where more than 80% spores germinated (Table 2).

The results of the present investigation indicate that both the alkaloids were highly effective against phytopathogenic and saprophytic fungi including a biotroph while alstovenine, a plant alkaloid from *Alstonia venenata*, was effective against pigmented and nonpigmented spores of several fungi. Conidia of *Erysiphe* sp. were very sensitive but spores of *Fusarium udum* were less sensitive to this alkaloid (Singh *et al.*, 1999). In the present investigation similar trend of efficacy was seen. *Erysiphe* sp. was quite sensitive to isocorypalmine. *H. penniseti* was more sensitive to (–)-corydalmine but *Alternaria* spp. were less sensitive against both the alkaloids. In a previous study, Singh *et al.* (1990) reported that hyaline spores were more sensitive to ajoene, a constituent of garlic (*Allium sativum*), as compared to pigmented ones. Maurya *et al.* (2001, 2002) reported that plant alkaloids are quite effective against both kinds of spores (pigmented and non-pigmented) belonging to different genera. The alkaloids used in the present study were also quite effective against both types of spores. Different groups of biochemicals are found in plant tissues in which some are antifungal, i.e. alkaloids, steroids, chalcones, phenolic acids etc. Alkaloids in general are reported to be highly inhibitory to fungal growth (Atta-ur-Rahman *et al.*, 1997; Bracher *et al.*, 1994: Liu *et al.*, 1990; Mahajan *et al.*, 1982; Singh *et*

Table 2. Effect of (–)-corydalmine on spore germination of some fungi

Fungus	Host	Percent spore germination (%) Concentration (μg/ml)					CD
		±	±-1	100	200	400	
Alternaria brassicae	*Brassica campestris*	93.00	93.00	96.00	94.00	4.00**	4.791
Alternaria solani	*Solanum tuberosum*	99.00	98.00	80.00**	9.00**	5.00**	3.159
Colletotrichum gloeosporioides	*Mangifera indica*	82.00	77.00	25.00**	0.00	0.00	13.677
Curvularia sp.	*Imperata cylendrica*	88.00	88.00	19.00**	5.00**	2.00**	11.724
Curvularia maculans	*Musa paradisica*	96.00	99.00	58.00**	0.00	0.00	11.631
Curvularia pinniseti	*Pennisetum typhoides*	88.00	91.00	3.00**	1.00**	0.00	5.139
Helminthosporium pinniseti	*Pennisetum typhoides*	96.00	96.00	83.00**	75.00**	0.00	4.786

**Shows data are significantly different at 1% level ($p \leq 0.01$).
± Control (water), ±-1 Control + MeOH, CD = Critical Deference.

al., 1994; Amir Basha *et al.*, 2002). The antifungal activity of the two compounds used in the present experiment is being reported for the first time. The high efficacy of these two plant alkaloids against in spore germination of some fungi shows a possibility of their use in controlling some plant diseases under field conditions.

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