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FUNGAL SPORE GERMINATION INHIBITION BY ALKALOIDS DEHYDROCORYDALMINE AND OXYBERBERINE

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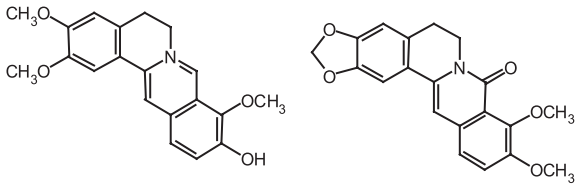
Abstract: The alkaloids dehydrocorydalmine and oxyberberine isolated from *Argemone mexicana* were assessed against spore germination of some fungi, e.g., *Alternaria cajani, Bipolaris* sp., *Helminthosporium* sp., *Fusarium udum* and *Curvularia* sp. While dehydrocorydalmine inhibited 100% spore germination of the fungi *Helminthosporium* sp. and *Curvularia* sp. at 5 000 ppm, oxyberberine showed similar activity against spore germination of *Bipolaris* sp. and *Curvularia* sp. All the five fungi were significantly inhibited at 1 000 to 5 000 ppm concentrations.

Key words: antifungal activity, dehydrocorydalmine, oxyberberine, *Argemone mexicana*

INTRODUCTION

The fungal diseases of plants have always been one of the major constrains in crop production causing severe losses every year. The indiscriminate use of various synthetic fungicides for the control of pests and diseases of crop plants for the past several decades has posed a serious threat to human health and environment leading to disturbed biodiversity, outbreaks of secondary pests, development of resistance in the pathogens and contamination of food chain in the ecosystem (Lyon *et al*. 1995). The farmers have used injudicious doses of synthetic fungicides for controlling plant diseases, giving rise negative effects. However, the researchers are on the way to finding out alternatives of synthetic fungicides. Eco-friendly systems involving biodegradable plant products from medicinal plants and biological agents (Prithiviraj and Singh 1996) which act directly or indirectly by inducing resistance in plants (Mishra and Raja 1999) and as fungicides have gained considerable importance as an alternative to synthetic fungicides. Several workers have used crude plant extracts *in vitro*, in glasshouse and field conditions against several plant pathogens (Vollekova *et al.* 2001; Prithiviraj *et al.* 1996). Various active principles isolated from the plants were proved effective against several plant pathogenic fungi *in vitro* (Singh *et al*. 1995; Singh *et al*. 2007).

The antifungal activity of a number of plant alkaloids have earlier been reported (Atta-ur Rahman *et al*. 1997; Sarma *et al.* 1999; Maurya *et al*. 2001; Maurya *et al*. 2002; Sahni *et al*. 2005; Basha *et al*. 2007). We report here for the first time the efficacy of dehydrocorydalmine (Fig. 1a) and oxyberberine (Fig. 1b) against spore germination of some plant pathogenic fungi.



Dehydrocorydalmine (a) Oxyberberine (b)

Fig. 1. Structure of dehydrocorydalmine and oxyberberine

MATERIALS AND METHODS

Argemone mexicana Linn. (Family *Papaveraceae*) is distributed throughout India, Nepal and Bangladesh. It is used in the Indian System of Medicine (Ayurveda) for the treatment of skin diseases, inflammations, fevers, diarrhoea and dysentery and also used as diuretic and purgative (Das and Khanna 1997). The plants were collected from Varanasi district, U.P., India. They were dried, powdered and extracted with methanol (MeOH) in a Soxhlet extractor. The extract was dried and extracted again with 7% aqueous citric acid. The acidic fraction was made alkaline with ammonia and extracted with $CHCl_3$. The alkaline solution left after extraction with $CHCl_3$ was acidified with dilute HCl and Mayer's reagent ($HgCl_2$

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+ KI) was added till complete precipitation. The precipitate was filtered and washed thoroughly with water. It was then mixed with water and stirred vigorously with IRA 410 (Cl^-) resin. The aqueous solution was separated from the resin by filtration and evaporated to dryness under reduced pressure, which furnished a brown semi-solid substance. It was chromatographed over silica gel column and eluted with solvents of increasing polarity. The $CHCl_3$-MeOH (50:1) and (8:1) eluates in crystallisation from MeOH furnished respectively the alkaloids, dehydrocorydalmine (Doskotch *et al*. 1967) as light yellow granules, m.p. 250–52°C, $C_{20}H_{20}NO_4$ ($[M]^+$ m/z 338.1405) (Fig. 1a) and oxyberberine (Chatterjee and Maiti 1955) as golden yellow needles, m.p. 198–200°C, $C_{20}H_{17}NO_5$ ($[M]^+$ m/z 351.1710) (Fig. 1b). These compounds were characterised by comparison of the spectral data, viz., IR, UV, ^1H and ^{13}C NMR, MS (Mass spectroscopy) with the reported data.

The fungi (Tables 1, 2) were isolated on Potato Dextrose Agar (PDA.) (peeled potatoes 250 g, dextrose 20 g, agar 15 g, distilled water 1 l) from their respective hosts collected from the experimental farm of the Banaras Hindu University, Varanasi, 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. Eight to ten-day old cultures were used in this experiment. The stock solution of 5 000 ppm of dehydrocorydalmine and oxyberberine were prepared separately by dissolving 5 mg of the chemical in a few drops of methanol followed by the addition of 1 ml of water. The methanol was evaporated on water bath and the concentration was maintained by further addition of water. Different concentrations, e.g. 1 000, 2 000, 3 000, 4 000 and 5 000 ppm were separately prepared for dehydrocorydalmine and oxyberberine by diluting the stock solution in sterile distilled water. One drop (30–35 µl) of each concentration was placed on a greese-free slides in which spores of the test fungus from the 8–10 day-old cultures were mixed. Spores of each fungus mixed only with sterile distilled water served as control. All the slides were then placed in moist chambers prepared by sticking wet filter paper inside the base and lid of sterile Petri dishes and incubated at 25±2°C for 24 h. After incubation, a drop of cotton blue prepared in lactophenol was added to spore suspension drop on each slide. Germinated spores of each test fungus in each concentration of dehydrocorydalmine and oxyberberine and in control slide were counted under bionocular light microscope and finally per cent spore germination was calculated. All the experiments were conducted in triplicate.

Table 1. Effect of dehydrocorydalmine on spore germination of some test fungi

Test Fungi	Tested concentration [ppm]						Critical Difference (CD)
	Control	1 000	2 000	3 000	4 000	5 000	
	% spore germination						
Alternaria cajani	92.02	78.28	64.42	50.24	32.66	11.74	14.26
Bipolaris sp.	91.77	77.02	64.08	48.38	31.26	10.15	13.57
Helminthosporium sp.	68.44	52.04	36.19	35.24	21.99	0	12.96
Fusarium udum	82.58	69.82	51.44	30.67	21.88	5.74	10.84
Curvularia sp.	52.17	41.96	28.42	14.28	0	0	11.86

Table 2. Effect of oxyberberine on spore germination of some test fungi

Test Fungi	Tested concentration [ppm]						Critical Difference (CD)
	Control	1 000	2 000	3 000	4 000	5 000	
	% spore germination						
Alternaria cajani	89.68	63.9	52.29	35.98	22.39	12.19	15.86
Bipolaris sp.	70.86	63.31	35.83	24.77	14.85	0	14.97
Helminthosporium sp.	72.24	57.66	40.49	36.64	15.4	7.82	13.56
Fusarium udum	87.71	65.53	45.85	32.75	21.51	5.42	12.64
Curvularia sp.	52.94	42.37	32.22	20.6	8.48	0	14.76

RESULTS

The alkaloid dehydrocorydalmine inhibited spore germination of the 5 fungal species under study and germination of all the fungi was significantly inhibited at all the concentrations used in this experiment. The spores of *Helminthosporium* sp. and *Curvularia* sp. did not germinate at all at 5000 ppm to which *Curvularia* sp. was highly sensitive at 4000 ppm. *Alternaria cajani*, *Bipolaris* sp. and *Fusarium udum* were slightly resistant to this compound as they showed 11.74%, 10.15% and 5.74% germination, respectively, even at 5 000 ppm (Table 1).

The alkaloid oxyberberine inhibited 100% spore germination of *Bipolaris* sp. and *Curvularia* sp. at 5000 ppm. The germination of all the five fungi was greatly inhibited at 1 000 to 4 000 ppm. *A. cajani*, *Helminthosporium* sp. and *F. udum* were slightly resistant at 5000 ppm (Table 2).

DISCUSSION

During the course of studies conducted by several workers to find out alternatives to synthetic chemical fungicides, a number of chemical compounds isolated from plants were found to be antifungal (Singh *et al.* 1995; Atta-ur Rehman *et al* 1997; Sarma *et al.* 1999; Maurya *et al.* 2001; Basha *et al.* 2007; Singh *et al.* 2007). Although several alkaloids are already known to be antifungal, the results of the present experiments also showed antifungal activity of the alkaloids, dehydrocorydalmine and oxyberberine at several concentrations. Testing of these compounds under field conditions against some fungal plant diseases may be interesting as their antifungal activity is being reported for the first time.

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POLISH SUMMARY

INHIBOWANIE KIEŁKOWANIA ZARODNIKÓW GRZYBÓW PRZEZ ALKALOIDY DEHYDROKORYDALMINĘ I OKSYBERBERYNĘ

Określano wpływ alkaloidów dehydrokorydalminy i oksyberberyny wyosobnionych z *Argemone mexicana* na kiełkowanie zarodników niektórych grzybów, w tym *Alternaria cajani, Bipolaris* sp., *Helminthosporium* sp., *Fusarium udum* i *Curvularia* sp. Podczas gdy dehydrokorydalmina inhibowała w 100% kiełkowanie zarodników grzybów *Helminthosporium* sp. i *Curvularia* sp. w stężeniu 5 000 ppm, oksyberberyna wykazywała podobną aktywność przeciwko kiełkowaniu zarodników *Biopolaris* sp. i *Curvularia* sp. Wszystkie pięć grzybów było istotnie inhibitowane w stężeniach od 1 000 do 5 000 ppm.