Mycobiology 29(2): 100-103 (2001)


# Antifungal Activity of Chaerophylline and Berberine Hydroxide Isolated from *Corydalis* Species

**Leepika Tuli, R. N. Jha[1], V. B. Pandey[1], and U. P. Singh***

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

**Chaerophylline and berberine hydroxide, both being alkaloids, isolated from *Corydalis chaerophylla* and *C. longipes* respectively were assayed against spore germination of some fungi, e.g. *Alternaria solani*, *A. brassicola*, *A. brassicicola*, *Helminthosporium penniseti*, *Helminthosporium* sp., *Heterosporium* sp., *Curvularia penniseti*, *C. maculens* and *C. palliscens*. While chaerophylline inhibited spore germination of most of the fungi at 1000 ppm, being also effective at 50, 100, 200 and 500 ppm, berberine hydroxide was significantly effective at much lower concentration, i.e., 400 ppm against several fungi. This compound was also effective against some fungi at 50, 100, 150, 200 ppm. There was 100% inhibition of spore germination in several fungi at highest concentration of both the compounds. Some of the fungi showed similar results even at lower concentrations.**

**KEYWORDS: Antifungal activity, Chaerophylline, Berberine, *Corydalis***

Fungal diseases have always been one of the major constraints in crop production causing severe losses each year. The damage caused by the various fungal diseases on crop plants entailed the usage of synthetic fungicides. Several effective fungicides have been synthesised which not only effectively controlled fungal diseases but also lowered considerable crop loss by resisting a narrow range of pathogens in a species. However, the side effects of these synthetic pesticides on human and animal health and also on the agroecosystem brought about research efforts on developing environment-friendly methods of controlling fungal diseases.

Some of these methods that have gained considerable importance are use of biocontrol agents (Vidyasekaran and Muthamilan, 1995; Vidyasekaran *et al.*, 1997), plant immunization (Kuc, 1987) or induced systemic resistance and use of natural plant products (Prithiviraj *et al.*, 1997a, b; Singh *et al.*, 1980, 1988). One of the several ecofriendly methods of controlling pests and diseases is the application of plant products in plant disease control. Many workers have screened plant products/extracts from higher plants for antifungal activity.

The success received from the use of plant extracts and plant products *in vitro* against plant pathogens led to the finding of possible antifungal compounds as substitutes of conventional fungicides that would provide effective disease control under field conditions minimizing environmental impacts also.

Various active principles isolated from plant extracts were found effective against plant pathogens and some of them responsible for antimicrobial activity are flavonoids, sioflavonoids, alkaloids etc. The antimicrobial activity of flavanoids and isoflavanoids has been extensively studied and established (Fraile *et al.*, 1982; Gnanmanickam and Smith, 1980; Weidenborner *et al.*, 1987; Vanetten, 1976; Skipp and Bailey, 1977; Perrin and Cruickshank, 1969). Many alkaloids with antimicrobial activity have been reported (Attaur-Rahman *et al.*, 1997; Bracher *et al.*, 1994; Liu *et al.*, 1990; Mahajan *et al.*, 1982; Mitcher *et al.*, 1975; Singh *et al.*, 1994; Srivastava *et al.*, 1994). The present study deals with the antifungal activity of chaerophylline and berberine hydroxide.

*Corydalis chaerophylla* Prodr. (Family : *Fumariaceae*) is distributed throughout Himalayan region and Nepal. Review of literature revealed that no medicinal use and isolation of chemical constituents has been reported from this plant. *Corydalis longipes* DC (Family : *Fumariaceae*) is distributed in Himalayas from Garhwal to Sikkim and Nepal. A number of isoquinoline alkaloids have earlier been reported from this plant without any evidence of medicinal use. Both chaerophylline and berberine hydroxide were used to evaluate their potential as an antifungal agent.

## Materials and Methods

The leaves of *C. chaerophylla* and *C. longipes* were dried, powdered and extracted with methanol in a Soxhlet extractor. The methanol extract was treated with 7% citric acid. The acidic solution was basified with $NH_4OH$ and further extracted with $CHCl_3$ which gave a mixture of crude base fraction on removal of the solvent. The crude base fraction of each plant was chromatographed over $SiO_2$ gel column eluting with solvents of increasing polar-

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

ity. In case of chaerophylline, the eluents collected from $CHCl_3$-MeOH (95 : 5) were mixed together according to TLC and crystallised from MeOH which furnished colourless granules of an alkaloid, Rf. 0.41 ($CHCl_3$-MeOH, 10 : 1), m.p. 173.74°C, molecular formula $C_{18}H_{21}NO_3$ ($M^+$ 299), whereas in case of berberine hydroxide the eluents from $CHCl_3$-MeOH (8 : 2), on crystallization from methanol furnished an alkaloid (67 mg) as yellow granules, Rf. 0.56 ($CHCl_3$-MeOH, 1 : 1), m.p. 145~147°C, $C_{20}H_{19}NO_5$ ($M^+$ 353). The molecular formula of each compound was settled by mass spectrum. The UV spectrum for chaerophylline showed absorption maxima at 284 and 254 nm like that of tetrahydroberberine. IR spectrum exhibited absorption maxima at 3300 $cm^{-1}$ for hydroxyl group. The $^1$HNMR, $^{13}$CNMR and mass spectral data were in favour of the structure of chaerophylline (Jha and Pandey, 2001) (Fig. 1) whereas berberine hydroxide exhibited UV absorption maxima at $\check{Z}_{max}$ 349, 265 and 231 nm like that of berberine. $^1$H and $^{13}$C NMR and mass spectra were identical with the reported data of berberine. It was finally identified as alkaloid, berberine hydroxide (our unpublished data) by direct comparison with authentic sample (mixed m.p., CO-TLC and superimposable IR) (Fig. 2).

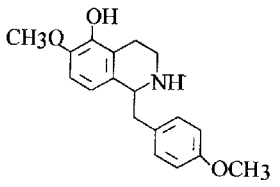


**Fig. 1.** Chaerophylline.

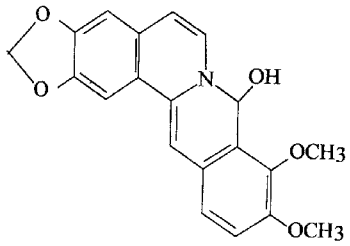


**Fig. 2.** Berberine hydroxide.

The fungi used for testing the antifungal activity of

**Table 1.** Effect of chaerophylline on spore germination of some fungi

| Fungus | Host | Concentration (ppm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Control | 50 | 100 | 200 | 500 | 1000 |
| | | Percent germination | | | | | |
| *Alternaria brassicola* | *Brassica campestris* | 85.22 | 83.26$^{NS}$ | 58.58** | 17.27** | 0.00** | 0.00** |
| *Alternaria solani* | *Solanum tuberosum* | 92.08 | 63.26** | 35.76** | 24.38** | 4.92** | 1.62** |
| *Curvularia lunata* | *Oryza sativa* | 86.8 | 9.80** | 8.20** | 0.00** | 0.00** | 0.00** |
| *Curvularia* sp. | *Sesamum indicum* | 95.28 | 47.95** | 11.33** | 0.99** | 0.31** | 0.26** |
| *Curvularia maculens* | *Musa sapientum* | 85.41 | 54.17** | 49.21** | 13.57** | 8.90** | 0.00** |
| *Curvularia penniseti* | *Pennisetum typhoides* | 94.03 | 86.35$^{NS}$ | 61.42** | 19.21** | 2.63** | 2.95** |
| *Helminthosporium* sp. | *Echinocloa* sp. | 88.76 | 21.73** | 20.61** | 7.03** | 0.00** | 0.00** |
| *Helminthosporium penniseti* | *Pennisetum typhoides* | 98.39 | 0.00** | 0.00** | 0.00** | 0.00** | 0.00** |

CD = 18.90.
Row data with ** varies significantly ($P \leq 0.01$).
NS - nonsignificant.

**Table 2.** Effect of chaerophylline on spore germination of some fungi

| Fungus | Host | Concentration (ppm) | | | | | |
|---|---|---|---|---|---|---|---|
| | | Control | 50 | 100 | 150 | 200 | 400 |
| | | Percent germination | | | | | |
| *Alternaria solani* | *Solanum tuberosum* | 93.52 | 55.58** | 38.56** | 14.88** | 11.75** | 0.64** |
| *Alternaria brassicola* | *Brassica campestris* | 94.83 | 71.51$^{NS}$ | 50.84** | 37.18** | 21.20** | 0.33** |
| *Alternaria brassicicola* | *Brassica oleracea* var. *capitata* | 81.55 | 79.50$^{NS}$ | 68.29** | 19.21** | 4.57** | 0.00** |
| *Helminthosporium penniseti* | *Pennisetum typhoides* | 88.46 | 82.11$^{NS}$ | 68.69** | 48.56** | 6.16** | 1.33** |
| *Helminthosporium* sp. | *Echinocloa* sp. | 97.44 | 64.68** | 35.62** | 12.22** | 7.26** | 0.00** |
| *Heterosporium* sp. | *Casia fistula* | 87.24 | 0.66** | 0.33** | 0.00** | 0.00** | 0.00** |
| *Curvularia penniseti* | *Pennisetum typhoides* | 96.15 | 67.99** | 1.29** | 1.33** | 0.33** | 0.00** |
| *Curvularia maculens* | *Musa sapientum* | 87.67 | 59.35** | 11.13** | 11.08** | 5.59** | 0.00** |
| *Curvularia palliscens* | *Bambusa indica* | 97.08 | 44.11** | 38.03** | 28.93** | 0.00** | 0.00** |

CD = 20.11.
Row data with ** vary significantly ($P < 0.01$).
NS = nonsignificant.

chaerophylline and berberine hydroxide were isolated from their respective hosts (Tables 1 and 2) on PDA (250 g peeled potato, 20 g dextrose, 15 g agar and 1 *l* distilled water) medium. The cultures were purified by single spore isolation on PDA slants and maintained at 25±2°C for experiments.

Stock solution of 5000 ppm of each compound was prepared separately by dissolving 5 mg of the chemical in minimal amount of methanol followed by the addition of 5 m*l* of water. The methanol was evaporated on water bath and different concentrations, e.g., 50, 100, 200, 500, 1000 ppm were prepared for the chaerophylline and 50, 100, 150, 200 and 400 ppm for berberine hydroxide, respectively, by diluting the stock solution in sterile distilled water. One drop (30~40 $\mu l$) from each concentration was placed on a grease-free slide in which spores of the test fungus from the 8-10-day-old cultures were mixed. Spores of each fungus mixed in sterile distilled water only served as control. All the slide were then placed in moist chambers prepared by sticking wet filter paper inside the base and the lid of the sterile petri dishes and incubated at 25±2°C for 24 h. After incubation the slides were fixed with cotton blue prepared in lactophenol. Germinated spores of each test fungus in each concentration of chaerophylline and berberine hydroxide were counted under a binocular light microscope and finally percent spore germination was calculated. All the experiments were conducted in triplicate. The data were subjected to Student's *t* test for statistical significance.

## Results and Discussion

Chaerophylline was highly effective in controlling spore germination of most of the fungi tested (Table 1). *H. penniseti* showed complete inhibition at the lowest concentration (50 ppm) whereas there was no germination in 200 and above concentrations in *C. lunata*. On the other hand, another species of *Helminthosporium* and *A. brassicola* showed 100% inhibition of spore germination at 500 ppm and onwards. *C. penniseti* was slightly resistant to this chemical as it showed germination (2.95%) even at 1000 ppm followed by *A. solani* and *Curvularia* species.

Berberine hydroxide was also highly effective in inhibiting spore germination of most of the fungi tested (Table 2). While there was no germination of conidia of *Heterosporium* species at 150 and above concentrations, the inhibition was highly significant even at 50 and 100 ppm as compared to control. Among *Alternaria* species, *A. solani* and *A. brassicola* showed slight germination at 400 ppm while there was significant inhibition at 50 and 100 ppm also. Similarly, *Helminthosporium* species were also significantly inhibited. There was no germination at maximum concentration (400 ppm) of *Curvularia* species but even at other concentrations, the germination was significantly checked. Similar inhibitory effect was seen for *C. palliscens*.

The results of the present experiments on the efficacy of berberine hydroxide isolated from *C. longipes* against nine fungal species which incite serious diseases in crop plants, exhibited *A. brassicola*, *Helminthosporium* sp., *Heterosporium* species, *C. pennistti*, *C. maculens* and *C. palliscens* as highly sensitive at the maximum concentration (400 ppm). However, among all these fungal species the chemical was significantly effective against *Heterosporium* species at all concentrations. In general, the chemical was effective against most of the fungi even at the lowest concentration (50 ppm) except *A. brassicicola* and *H. penniseti*. Interestingly both the chemicals were highly effective against all the fungi tested, it would be of interest to assess their efficacy against some important crop plant diseases under field conditions. Berberine hydroxide isolated for the first time from *C. longipes* has given much better result than chaerophylline, hence it is envisaged that it may prove much better than the conventional synthetic fungicides in plant disease control, in the field.

## References

Atta-Ur-Rahman, A. Nasreen, Akhtar, F., Shekhani, M. S., Clardy, J., Parvez, M. and Choudhary, M. I. 1997. Antifungal diterpenoids alkaloids from *Delphinium denudatum. J. Nat. Prod.* **60**: 474-474.

Bracher, E. 1994. Polycyclic aromatic alkaloids. 10. Annonaceous alkaloids with antimycotic activity. *Arch. Pharm. Weinheim* **327**: 371-375.

Fraile, A., Garcia-Arenal, F., Garcia-Serano, J. J. and Sagasta, E. M. 1982. Toxicity of phaseollin, phaseollidin, phaseollinisoflavan and kievitone to *Botrytis cinerea. Phyopathol. Z.* **105**: 161-169.

Gnanmanickam, S. S. and Smith, D. A. 1980. Selective toxicity of isoflavonoid phytoalexins to Gram positive bacteria. *Phytopathology* **70**: 894-896.

Kuc, J. 1987. Plant immunization and its applicability for disease control, pp. 225-274. John Wiley, New York.

Liu, S. C., Oguntimin, B., Hufford, C. D. and Clark, A. M. 1990. 30Methoxy-sampangine, a novel antifungal copyrine alkaloid from *Cleistopholis putens. Antimicrob. Agents Chemother.* **34**: 529-533.

Mahajan, V. M., Sharma, A. and Rattan, A. 1982. Antimycotic acitivity of berberine sulphate : an alkaloid from an Indian medicinal herb. *Sabouaudia* **20**: 79-81.

Mitcher, L. A., Bathala, M. S., Clark, G. W. and Beal, J. L. 1975. Antimicrobial agents from higher plants. The quaternary alkaloids of Ptelea trifoliata. *Lloydia.* **38**: 109-116.

Perrin, D. R. and Cruickshank, I. A. M. 1969. The antifungal activity of pterocarpans towards *Monilia fructicola. Phytochemistry* **8**: 971-978.

Prithiviraj, B., Manickam, M., Singh, U. P. and Ray, A. B. 1997a. antifungal activity of anacardic acid, a naturally occurring derivative of salicylic acid. *Can. J. Bot.* **75**: 207-211.

Prithiviraj, B., Singh, U. P., Manickam, M., Srivastava, J. S. and Ray, A. B. 1997b. Antifungal acitivity of ergenin, a constituent

of *Flueggia microcarpa. Plant Pathol.* **46**: 244-248.

Singh, U. P., Singh, H. B. and Singh, R. B. 1980. The fungicidal effect of neem (*Azadirachta indica*) on some soil-borne pathogens of gram (*Cicer arietinum*). *Mycologia* **72**: 1077-1093.

_____, Pandey, V. B., Singh, K. N. and Singh, R. D. N. 1988. Antifungal activity of some new flavonones and flavone glycosides of *Echinops echinatus. Can. J. Bot.* **66**: 1901-1903.

_____, Singh, K. P., Tripathi, V. K. and Pandey, V. B. 1994. Antifungal activity of some naturally occurring plant alkaloids. *Int. J. Trop. Plant Dis.* **12**: 209-212.

Skipp, R. A. and Bailey, J. A. 1977. The fungitoxicity of isoflavonoid phytoalexins measured using different types of bioassay. *Physiol. Plant Pathol.* **11**: 101-112.

Srivastava, B. P., Singh, K. P., Singh, U. P. and Pandey, V. B. 1994. Effect of some naturally occurring alkaloids on conidial germination of *Botrytis cinerea. Bioved* **5**: 69-72.

Van Etten, H. D. 1976. Antifungal activity of ptercarpans and other selected isoflavonoids. *Phytochemistry* **15**: 655-659.

Vidyasekaran, P. and Muthamilan, M. 1995. Development of formulation of *Pseudomonas flourescens* for control of chickpea wilt. *Plant Dis.* **79**: 782-786.

_____, Sethuraman, K., Rajappan, K. and Vasumathi, K. 1997. Powder formulation of *Pseudomonas flourescens* to control pigeon pea wilt. *Biolog. Contr.* **8**: 166-171.

Weidborner, M., Jha, H. C., Hindorf, H., Weltizien, H. C. and Ziliken, F. 1987. Wirkung van isolavonoide auf schimmelpilze der gattung *Aspergillus. Med. Fac. Landbouww. Rijksuniv. Gent.* **52**: 933-942.