

Inhibitive Effect of Fuyuziphine isolated from Plant (Pittapapra) (*Fumaria indica*) on Spore Germination of Some Fungi

M. B. Pandey¹, Ashok K. Singh^{2*}, Anil K. Singh³ and U. P. Singh⁴

¹Department of Chemistry, S.G.R.P.G. College, Dobhi - 222149 (Purvanchal University) Jaunpur, India

²Department of Medicinal Chemistry, Institute of Medical Sciences, Banaras Hindu University, Varanasi - 221005, India

³Department of Dravya Guna, Institute of Medical Sciences, Banaras Hindu University, Varanasi - 221005, India

⁴Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi - 221005, India

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The alkaloid fuyuziphine was isolated from the whole plant of *Fumaria indica*. It had inhibitive effect against spore germination of some plant pathogenic fungi (*Colletotrichum* sp., *C. gloeosporioides*, *C. falcatum*, *Curvularia maculans*, *C. lunata*, *Erysiphe cichoracearum*, *Helminthosporium pennisetii*, *Oidium erysiphoides*, *Ustilago cynodontis*, *Alternaria chieranthi*, *A. melongenae*, *A. brassicicola* and *A. solani*). *Curvularia lunata*, *Oidium erysiphoides*, *Alternaria brassicicola* and *A. solani* did not germinate at 750 and 1000 ppm and *Colletotrichum gloeosporioides*, *C. falcatum*, *Curvularia maculans* were inhibited at 1000 ppm for 24 hr incubation. Germination of most fungi was significantly inhibited at 100~750 ppm.

KEYWORDS: *Fumaria indica*, Fuyuziphine, Inhibition of spore germination

Due to growing interest for the search of naturally occurring substances as anti-fungal agent in agricultural production, considerable work has been done on plant extracts (Prithiviraj *et al.*, 1996) and it has been reported their various constituents have antifungal activity against phytopathogenic fungi *in vitro* (Maurya *et al.*, 2002), green house (Singh *et al.*, 1995) and in open field (Prithiviraj *et al.*, 1998). Several plant alkaloids have been known to affect biological functions of microbes at very low concentrations (Basha *et al.*, 2002; Sahni *et al.*, 2005).

In this study, we report the antifungal activity of the alkaloid, fuyuziphine isolated from the whole plant of *Fumaria indica* which the antifungal activity of this alkaloid has not yet been reported to our knowledge.

The plant *Fumaria indica* (Fumariaceae) is distributed over greater parts of India and used in Indian System of medicine as laxative, diuretic and is beneficial in dyspepsia and liver complaints (Chopra *et al.*, 1956). The plant was collected from Mirzapur District, U.P., India, dried in sunlight and powdered mechanically. The powdered plant was extracted with MeOH in a Soxhlet extractor. The extract was dried *in vacuo* and extracted again with 7% aqueous citric acid. The acidic fraction was alkalized with ammonia and extracted with CHCl₃. The CHCl₃ extract was concentrated and chromatographed over silica gel column eluting with solvents of increasing polarity. The eluates from CHCl₃-MeOH (9 : 1) were crystallized in MeOH and furnished an alkaloid as colourless amorphous powder. It was characterised by a study of the

detailed spectroscopic data i.e. IR, UV, ¹H NMR, ¹³C NMR and MS spectra and chemical evidences (Pandey *et al.*, 2007).

The plant pathogenic fungi (Table 1) were isolated from their respective hosts in the experimental farm of the Banaras Hindu University, Varanasi, India. Seven to ten days old cultures on PDA were used in this experiment. The spores of obligate parasitic fungi were directly picked up from their respective hosts. The stock solution (2000 ppm) was prepared by dissolving 10 mg of the alkaloid fuyuziphine initially with a few drops of methanol in a test tube. After the chemical was completely dissolved, approximately 5 ml of distilled water was added. The solvent methanol was then evaporated on water bath. The required concentration (100, 250, 500, 750, and 1000 ppm) of the chemical were prepared from the stock solution by diluting with distilled water. A drop (30~40 ml) of the fuyuziphine 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. 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 hr for germination. The germination of the spores was observed after staining with cotton blue. All the experiments were conducted in triplicate.

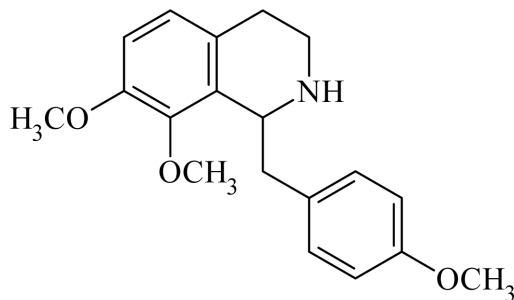
In this study, the alkaloid fuyuziphine (Table 1) (100~1000 ppm) affected the spore germination of the 13 fungal species. The spore germination of all the fungi was inhibited at 1250 ppm. Spores of *Colletotrichum gloeosporioides*, *C. falcatum*, *Curvularia maculans*, *C. lunata*, *Oid-*

*Corresponding author <E-mail: singhak06@rediffmail.com>

Table 1. Inhibitive effect of fuyuziphine (100~1000 ppm) on spore germination (%) of some fungi^a

Fungus	% of spore germination according to concentration (ppm)					
	0	100	250	500	750	1000
<i>Alternaria chieranthi</i>	78.4	48.4**	31.0**	24.5**	21.5**	12.2**
<i>Alternaria brassicicola</i>	45.4	20.0**	15.5**	9.5**	0**	0**
<i>Alternaria melongenae</i>	64.8	30.8**	27.0**	15.0**	30.0**	2.3**
<i>Alternaria solani</i>	66.4	20.0**	15.0**	2.0**	0**	0**
<i>Colletorichum falcatum</i>	96.4	63.0**	45.7**	24.0**	9.5**	0**
<i>Colletorichum gloeosporioides</i>	91.0	70.0**	55.2**	45.0**	12.2**	0**
<i>Colletorichum sp.</i>	89.0	60.0**	47.2**	25.5**	20.0**	15.0**
<i>Curvularia lunata</i>	91.2	40.4**	30.0**	20.0**	0**	0**
<i>Curvularia maculans</i>	94.2	70.0**	55.0**	23.0**	15.0**	0**
<i>Erysiphe cichoracearum</i>	58.7	42.2**	21.3**	11.5**	4.3**	3.0**
<i>Helminthosporium pennisetii</i>	68.5	18.0**	11.5**	8.5**	6.5**	2.3**
<i>Oidium erysiphoides</i>	89.8	60.0**	30.0**	26.3**	0**	0**
<i>Ustilago cynodontis</i>	85.5	40.0**	30.3**	20.5**	14.0**	3.2**

^aSpore germination was completely inhibited ($p < 0.01$) at 1250 ppm; ** $p < 0.01$.
The assay was carried out for 24 hr culture.

**Fig. 1.** Structure of Fuyuziphine.

ium erysiphoides, *Alternaria brassicicola* and *A. solani* did not germinate at 1000 ppm; *Curvularia lunata*, *Oidium erysiphoides*, *Alternaria brassicicola* and *A. solani* were inhibited also at 750 ppm. Germination of most of the fungi was significantly inhibited at 100~750 ppm.

We have previously reported the antifungal activity of the alkaloids protopine, tetrahydrocoptisine, narlumidine, fumariline, norsanguinarine (Singh *et al.*, 1997) and berberine iodide (Sarma *et al.*, 1999) isolated from *Fumaria indica*. This is the first report of the antifungal activity of fuyuziphine isolated from *F. indica*. The efficacy of fuyuziphine is significantly high even at low concentration which indicates a possibility of its use to control plant diseases under field condition.

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