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Antifungal Activity of Narceine Methyl Ester and Narceine Isolated from *Corydalis longipes* Against Some Phytopathogenic Fungi

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Narceine methyl ester and narceine are potent alkaloids which were isolated from *Corydalis longipes* were found effective *in vitro* at very low concentration, i.e., 100~500 ppm against spore germination of some test plant pathogenic fungi (*Alternaria solani*, *A. tagetica*, *Cercospora abelmoschi*, *Curvularia maculans*, *Erysiphe cichoracearum*, *E. pisi*, *Fusarium udum*, *Helminthosporium oryzae*, *H. penniseti*, *Ustilago cynodontis*). Among the test, phytopathogens the spores of *F. udum*, *C. maculans* and *H. penniseti* were highly sensitive at 200 ppm. However, spores of *E. pisi*, *A. solani* and *A. tagetica* were less sensitive at low concentration followed by other test fungi. Most of the fungi showed zero or nearly zero percent spore germination at 400 and 500 ppm.

KEYWORDS: Antifungal activity, *Corydalis longipes*, Narceine methyl ester, Narceine, Spore germination

From the very beginning of crop cultivation attempts have been made to manage fungal diseases of crop plants initially by worshiping the God and later by using synthetic fungicides (Agrios, 1997). Albeit the performance of several synthetic fungicides are phenomenal but their negative impact on living organisms and agro-ecosystem brought about changes in thinking for developing alternatives of plant disease management. They are biological control, induction of systemic acquired resistance (SAR) (Lyon *et al.*, 1995) and use of plant products of medicinal origin (Chakravorthy and Pariya, 1977; Mahajan *et al.*, 1982). Plant products have also been found to be effective under field condition (Singh *et al.*, 1995; Prithiviraj *et al.*, 1998). Antifungal activity of a good number of plant products (eg. alkaloids, flavonoides, terpenes, ajoene, etc.) has been reported by several workers (Mitcher *et al.*, 1975; Millard *et al.*, 1987; Liu *et al.*, 1990; Reimers *et al.*, 1993; Singh *et al.*, 1988, 1990, 1995, 2000; Maurya *et al.*, 2001; Basha *et al.*, 2002). Seed extract of *Trachispermum ammi* has been found to reduce the disease incidence of sheath blight of rice (*Rhizoctonia solani*) by 72.25 percent (Ansari, 1995).

Alkaloids even at very low concentrations often exhibit antimicrobial activity (Mitcher *et al.*, 1975; Bracher, 1994; Atta-ur-Rahman *et al.*, 1982; Singh *et al.*, 2000; Maurya *et al.*, 2002). Antifungal activity of berberine and (±) bicuculline, isolated form *Corydalis chaerophylla* has recently been reported effective against spore germination of some plant pathogenic fungi (Basha *et al.*, 2002). This study was conducted to see the efficacy of the alkaloids narceine methyl ester and narceine, which were isolated from *Corydalis longipes*, against spore germination of some plant pathogenic fungi.

Materials and Methods

Plant sample. *Corydalis longipes* Rodr. (Fumariaceae) was collected in Nepal. The whole plant was dried and extracted with methanol in a Soxhlet extractor. The extract was stirred with 7% citric acid. The acid fraction was alkalized with NH_4OH and extracted with chloroform. The extract was concentrated and chromatographed over silica gel column eluted with $CHCl_3$-MeOH mixture of increasing polarity.

Narceine methyl ester. The elutes from $CHCl_3$-MeOH (98:2) on crystallization from methanol furnished alkaloids as colorless needles, mp112~114°C. The molecular formula was determined as $C_{24}H_{29}NO_8$ (M^+, 459.1435) from its high-resolution mass spectrum. The UV spectrum showed maximum absorbance at wavelength 204, 227, 274 and 290 nm. IR, ^{1}HNMR and ^{13}CNMR were identical with the data reported for narceine (Blasko *et al.*, 1982) except the signals due to methyl ester group. Finally, the compound was identified as narceine methyl ester after comparison with an authentic sample (Narcein methyl ester) in all respects (mixed mp, co-TLC and super imposable IR).

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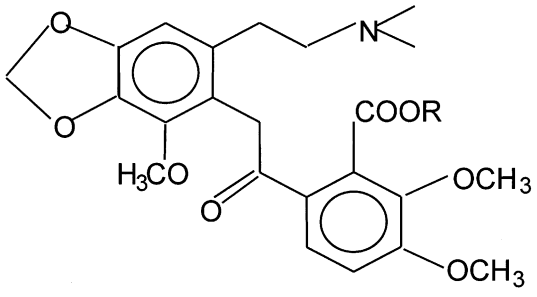

Narceine Methyl Ester R = CH_3

Narceine R= H

Fig. 1. Structure of narceine methyl ester and narceine.

Narceine. The eluates from $CHCl_3$-MeOH (8 : 2) on crystallization from methanol furnished an alkaloid as amorphous powder. The molecular formula was determined as $C_{23}H_{27}NO_8$ (M^+445). UV spectra were similar to compound narceine methyl ester. IR spectra were also similar with absence of methyl ester group at 1730 cm^{-1}. The above data together with chemical shift in ^{1}HNMR and ^{13}CNMR were identical to the reported data of narceine (Blasko *et al.*, 1982). The alkaloid was characterized finally as narceine by direct comparison with an authentic sample (mixed mp, co-TLC and super imposable IR).

Stock solution (600 ppm) was prepared by dissolving 3 mg of chemical initially with a few drops of methanol in a clean test tube. After the chemical was completely dissolved, approximately 5 *ml* of sterilized distilled water was added. The methanol was then evaporated on water bath (80°C). The required concentrations (100, 200, 300, 400 and 500 ppm) of the chemical were prepared from the stock solution by diluting with distilled water.

The test fungi were isolated on PDA (peeled potato 250 g, dextrose 20 g, agar 20 g, distilled water 1000 *ml*) from their respective hosts. The cultures were further purified by single spore isolation technique on PDA slant (Singh *et al.*, 1990). Seven to ten days old cultures were used in this experiment. The spores of obligate parasitic fungi were picked up directly from their respective hosts for this experiment.

A drop (30 μl) of chemical solution was placed on a grease free glass slide. Fungal spores were mixed (about 200~300) in the solution with the help of sterile inoculation needle. The conidia of obligate phytopathogenic fungi, *Ustilago* sp. and *Erysiphe* sp. were directly picked up from the diseased plant and mixed in the solution. The slides were later placed in moist chamber made by placing two sterile moist filter papers on the lid and base of Petri dishes. The spores were then incubated at 25±2°C for 24 hours for germination. Germination percentages of the spores were calculated after staining with cotton blue prepared in lacto phenol under binocular light microscope (Nikon, Japan). All the experiments were carried out in triplicate. The statistical analyses of the data were subjected to Student's-t test.

Results and Discussion

Narceine methyl ester was excellent in controlling spore germination of most of the fungi under study (Table 1). While there was no germination of spores of *F. udum* at 300 ppm and above, the inhibition was markedly significant even at 100 and 200 ppm. Similar inhibitory effect was seen for *E. cichoracearum* as well as *U. cynodontis.* Like other fungi in the present experiment, spore germination of *A. solani* and *A. tagetica* was significantly inhibited at 100~500 ppm but both showed least sensitivity at 100 ppm. Exceptionally, *H. oryzae* showed high resistance at 100 ppm and a dose of 400 ppm was required for complete inhibition of spore germination. On the other hand, germination of another species of *Helminthosporium* was significantly inhibited at 100 ppm. Nearly 100 percent inhibition was observed in *A. solani*, *A. tagetica*,

Table 1. Effect of Narceine methyl ester on spore germination of some fungi

Fungus	Host	Concentration (ppm)							
		C	M + W	100	200	300	400	500	CD at 1%
		Percent germination							
Alternaria solani	*Solanum tuberosum*	98.3	97.3	89.0**	1.3**	0.6**	0**	0**	3.02
A. tagetica	*Tagetes erecta*	98.6	97.3	49.0**	2.0**	0.6**	0**	0**	10.09
Cercospora abelmoschi	*Abelmoschus esculentus*	100.0	98.0	4.3**	1.3**	0.3**	0**	0**	3.02
Curvularia maculans	*Musa sapientum*	96.0	93.3	2.3**	1.3**	0.2**	0**	0**	4.27
Erysiphe cichoracearum	*Impatiens balsamina*	52.3	51.6	1.0**	0.6**	0**	0**	0**	8.18
E. pisi	*Pisum sativum*	52.6	46.6	11.0**	2.6**	0.6**	0**	0**	9.31
Fusarium udum	*Cajanus cajan*	95.3	95.0	1.0**	0.3**	0**	0**	0**	1.2
Helminthosporium oryzae	*Oryza sativa*	63.3	61.3	59.6	16.6**	6.0**	0**	0**	11.5
H. penniseti	*Pennisetum typhoides*	97.3	96.3	4.6**	2.3**	1.0**	0.3**	0.3**	3.7
Ustilago cynodontis	*Cynodon dactylon*	99.0	97.6	1.6**	0.6**	0**	0**	0**	2.13

**Values vary significantly ($p<0.01$), C.D. = Critical Difference, C = Control, M + W = Methanol + Water.

Table 2. Effect of Narceine on spore germination of some fungi

Fungus	Host	Concentration (ppm)							
		C	M + W	100	200	300	400	500	CD at 1 %
		Percent germination							
Alternaria solani	*Solanum tuberosum*	94.0	93.0	44.6**	20.6**	10.0**	2.3**	0**	8.64
A. tagetica	*Tagetes erecta*	97.6	96.6	9.6**	8.0**	3.0**	2.3**	1.2**	5.18
Curvularia maculans	*Musa sapientum*	92.6	90.3	11.6**	1.6**	0.3**	0**	0**	4.39
Erysiphe cichoracearum	*Impatiens balsamina*	52.6	51.0	17.6**	12.5**	5.6**	4.3**	2.5**	10.6
E. pisi	*Pisum sativum*	51.3	48.6	3.3**	2.3**	2.3**	1.3**	1.3**	4.83
Fusarium udum	*Cajanus cajan*	94.0	92.6	0.6**	0**	0**	0**	0**	2.94
Helminthosporium oryzae	*Oryza sativa*	49.5	47.6	20.4**	9.0**	2.3**	0**	0**	6.83
Ustilago cynodontis	*Cynodon dactylon*	92.0	91.0	6.3**	4.9**	1.6**	0.6**	0**	3.37

**Values vary significantly ($p<0.01$), C.D. = Critical Difference, C = Control, M + W = Methanol + Water.

C. abelmoschi, *C. maculans* and *E. pisi* at 300 ppm. Results showed that 300, 400 and 500 ppm concentrations were almost equally effective against most of the fungi tested.

Narceine was also effective against spore germination of all the eight fungal species (Table 2). The response of different fungi varied considerably. *F. udum* showed high sensitivity even at the lowest concentration (0.6 percent germination) and at 200 ppm and onward doses 100 percent inhibition was observed. Albeit, *C. maculans* and *H. oryzae* showed complete inhibition at 400 ppm but they were highly sensitive even at 200 and 300 ppm. Among the tested fungi, two species of *Erysiphe* were slightly resistant against the chemicals as they showed germination even at 500 ppm (2.5 percent in *E. cichoracearum* and 1.3 percent in *E. pisi*) followed by *A. tagetica* (1.2 percent). A concentration of 700 ppm and above was needed for their 100 percent inhibition.

Interestingly both the chemicals were highly effective against all the tested fungi even at very low concentration. Both hyaline and pigmented fungal spores showed almost similar sensitivity against the test alkaloids. Although several alkaloids have been reported to be antifungal including berberine hydroxide isolated from *Corydalis longipes* (Tuli *et al.*, 2001), the antifungal activity of narceine methyl ester and narceine isolated from the same plant species is being reported for the first time with greater efficacy. Moreover, the results are quite similar with the currently used synthetic fungicides like triademefon (100 ppm) and mancozeb (250 ppm) that showed effective result against *Erysiphe polygoni* (Gupta and Shyam, 1998). As most of the plant pathogenic fungi have shown high sensitivity against the chemicals, it may be interesting to use them under field conditions for managing fungal diseases of crop plants.

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