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Effect of Ent-norsecurinine, an Alkaloid, on Spore Germination of Some Fungi

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The inhibitory activity of ent-norsecurinine alkaloid was evaluated against spore germination of some plant pathogenic fungi (*Curvularia maculans*, *Curvularia* species, *C. palliscens*, *Colletotrichum gloeosporioides*, *Colletotrichum* species, *Alternaria solani*, *A. brassicae*, *Fusarium udum*, *Helminthosporium echinoclova* and *H. penniseti*). It inhibited spore germination of all the test fungi. *C. maculans*, *C.* species, and *C. palliscens* were the most sensitive as complete inhibition of spore germination was observed at 1000 ppm. *A. solani* was not inhibited by this chemical.

KEYWORDS: Alkaloid, Antifungal activity, Ent-norsecurinine, Spore germination

Increased agricultural production is attributed to excessive use of synthetic chemicals during the last century. This has raised a number of ecological and human health problems as most of them are associated with several harmful effects, *e.g.*, prolonged existence of these chemicals in the environment leads to resurgence of resistance among various fungi, and contaminates environment and food chain, which cause serious ecological imbalance. Increasing awareness of possible deleterious effects of fungicides on the ecosystem and growing interest in pesticide-free agricultural products have led to diversion of attention towards environment friendly approaches, such as genetic engineering for evolving resistant varieties and use of induced resistance by biotic and abiotic means (Lyon *et al.*, 1995). Drawbacks associated with synthetic fungicides have given way to think of the biodegradable plant products, especially from medicinal plants as their alternative (Basha *et al.*, 2002; Chaturvedi *et al.*, 1987; Khurana *et al.*, 1972; Maillard *et al.*, 1989; Maurya *et al.*, 2002; Prithiviraj *et al.*, 1995, 1996, 1998; Singh *et al.*, 1990, 2001; Tripathi *et al.*, 1983).

Plant extracts (Asthana *et al.*, 1982; Prithiviraj *et al.*, 1996; Vollekova *et al.*, 2001) and their various active constituents were found to be effective against phytopathogenic fungi *in vitro* (Maillard *et al.*, 1989; Maurya *et al.*, 2001, 2002; Prithiviraj *et al.*, 1997; Singh *et al.*, 1990), in greenhouse (Reimer *et al.*, 1993; Singh *et al.*, 1995) as well as in the field (Prithiviraj *et al.*, 1998; Sarma *et al.*, 1999; Smith *et al.*, 1983; Sriobaite, 1960). Several plant alkaloids are known to affect biological functions of microbes at very low concentrations (Bracher, 1994; Singh *et al.*, 1999, 2000; Srivastava *et al.*, 1994).

The plant *Phyllanthus amarus* (Euphorbiaceae) is distributed throughout India. Besides other uses, it is used widely as anti-hepatotoxic agent in Indian system of medicine (Ayurveda). The present study deals with the effect of some ent-norsecurinine, an alkaloid on spore germination of phytopathogenic and saprophytic fungi.

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Materials and Methods

The test fungi were isolated from their respective hosts collected from the experimental farm of the Banaras Hindu University (Varanasi, India) on potato dextrose agar (peeled potato 250 g, dextrose 20 g, agar 15 g, distilled water; 1 *l*). The cultures were further purified by single spore isolation technique and maintained at 25±2°C on PDA slants. Seven to ten-day-old cultures were used in the experiment.

The *Phyllanthus amarus* (Euphorbiaceae) is distributed throughout India. Dried whole plants (2.5 kg) were extracted successively with petroleum ether (60~80°), chloroform and methanol. The chloroform extract (50 g) was extracted with 7% aqueous citric acid by stirring mechanically four times. The combined acidic solution was basified with ammonium hydroxide and extracted in a separating funnel with chloroform. The chloroform extract was evaporated to dryness and chromatographed over silica gel column eluting with solvents of increasing polarity. The eluants collected from $CHCl_3$-MeOH (9 : 1) were mixed together according to TLC, which furnished pale yellow oil and did not crystallise with any solvents. The oil exhibited single spot on TLC plate having Rf 0.26 ($CHCl_3$-MeOH, 9.7 : 0.3). The oil was taken in methanol and ethereal HCl was added to it. After work up, it gave a crystalline hydrochloride. This was recrystallised from MeOH to give colourless plates, m. p. 270~272°C. $[\alpha]_D^{20}$ + 189° (c, 0.8, H_2O). The molecular formula was determined as $C_{12}H_{13}NO_2$ from its high-resolution mass spectral analysis (M^+, 203.1240). It exhibited UV (MeOH) having wavelength 256 nm and IR (KBr) exhibited absorp-

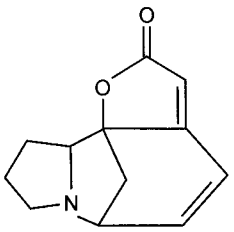

Fig. 1. Structural formula of ent-norsecurinine.

tion maxima at 10,805, 10,770 cm^{-1}, which was due to the presence of α, β-unsaturated-γ-lactone ring in the moiety. The above data together with ^{1}HNMR and ^{13}CNMR data were identical to the reported data of ent-norsecurinine. It was finally identified by comparison with authentic sample (mixed m. p., co-TLC and superimposable IR), (Joshi *et al.*, 1986) as ent-norsecurinine (Fig. 1).

Stock solution of ent-norsecurinine (1,000 ppm) was prepared by dissolving 5 mg of compound initially in a few drops of chloroform and methanol (1 : 1) in a test tube. After the chemical was completely dissolved, 5 m*l* of distilled water was added and solvents (chloroform and methanol) were evaporated on a water bath. Required concentrations (200, 400, 600, 800, 1,000 ppm) were prepared from the stock solution by diluting with distilled water. One drop (40 μl) from each concentration was placed on grease-free glass slide. Fungal spores (200~300) were picked up from 7-10-day-old growing cultures with a sterile inoculation needle and mixed in the solutions of chemical of different concentration separately. The slides were later placed in a 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 h. Germination was observed after staining with cotton blue prepared in lactophenol under binocular light microscope (*Nikon,* Japan Type 102). Spores mixed in sterile distilled water served as control. Other control having solvents ($CHCl_3$ and MeOH) in a 1 : 1 ratio was also kept. All the experiments were conducted in triplicate.

Results and Discussion

The effect of ent-norsecurinine on spore germination of some plant pathogenic fungi was seen (Table 1). The sensitivity of different fungi to this chemical varied considerably. Conidial germination of *C. maculans*, *Curvularia* species and *C. palliscens* was 0, 0.3 and 0.3%, respectively, at 800 ppm. Almost similar effect was recorded on *C. gloeosporioides* and *F. udum,* which showed 3.83 and 0.6% germination, respectively, at 1,000 ppm. However, *A. solani* was not inhibited completely as 12.33% germination at 1,000 ppm, which was highest amongst all the test fungi observed. Germination of spores of *H. echinoclova* and *H. penniseti* was recorded as 6.83 and 4.66%, respectively, and the germination of all the three species of *Curvularia* mentioned above was completely inhibited at the same concentration showing their highest sensitivity to the chemical. Thus, only high concentrations, *i. e.*, 800 and 1,000 ppm, were effective against most of the fungi.

The presence or absence of spore pigments did not seem to affect the activity of the chemical. Hyaline spores of *C. gloeosporioides* as well as pigmented ones of *Curvularia* and *Helminthosporium* species were equally sensitive to this chemical. Singh *et al.* (1990) found that hyaline spores of some fungi were more sensitive to ajoene, a compound isolated from garlic (*Allium sativum*), as compared to pigmented ones. Although several alkaloids are already known to be antifungal, e.g., (−)-corypalmine completely inhibited the spore germination of *C. maculans* at 200, 400, 600, 800 and 1,000 ppm and *C. palliscens* and *Curvularia* species were inhibited at 400 ppm (Maurya *et al.*, 2002). The present compound, ent-norsecurinine, completely inhibited the germination of all the above mentioned fungi only at 1,000 ppm. Singh *et al.*

Table 1. Effect of ent-norsecurinine on spore germination of some fungi

Fungus	Host	Concentration (ppm)							Critical Difference (CD)
		Percent spore germination							
		C	C + M	200	400	600	800	1,000	
Curvularia species	*Imperata cylendrica*	94.16	92.83	61.50**	8.50**	2.33**	0.30**	0.00**	15.32
C. maculans	*Musa paradisiaca*	94.00	92.83	73.83	55.50**	0.30**	0.00**	0.00**	20.48
C. palliscens	*Bombusa indica*	94.66	91.66	57.66**	10.50**	6.50**	0.30**	0.00**	16.11
Colletotrichum species	*Arundinaria falcata*	93.33	91.83	83.50**	78.16**	24.00**	17.66**	10.16**	7.36
C. gloeosporioides	*Mangifera indica*	97.83	94.50	91.16	66.33**	48.33**	15.33**	3.83**	21.64
Alternaria brassicae	*Brassica campestris*	92.50	92.16	80.83	72.00**	52.83**	43.50**	9.16**	17.15
A. solani	*Solanum tuberosum*	92.33	92.66	81.16	72.00**	60.33**	35.66**	12.33**	18.61
Fusarium udum	*Cajanus cajan*	79.83	75.33	53.66**	43.66**	12.83**	6.00**	0.60**	8.50
Helminthosporium echinoclova	*Echinoclova* species	97.50	92.33	80.16**	43.00**	20.00**	12.00**	6.83**	13.09
H. penniseti	*Pennisetum typhoides*	94.33	90.83	53.83**	50.83**	22.66**	18.00**	4.66**	10.85

C = Distilled water, C + M = Distilled water + few drops of methanol, ** Asterisk indicates that data were significantly different as compared to control values at p = 0.01 based on student t-test, CD = Critical Difference.

(2000) reported efficacy of venenatine, another alkaloid, which reduced germination of *F. udum* and *A. brassicae* to below 10% at 2 mg/*l* concentration whereas ent-norsecurinine showed spore germination of *A. brassicae* and *F. udum*, as 9.16 and 0.6% at 1,000 ppm, respectively. Singh *et al.* (2001) reported germination of *F. udum* and *A. brassicae* by 3.1 and 4.9%, respectively, when treated with berberine at 1,500 ppm (an isoquinoline alkaloid from *Fumaria indica*), the amount of which was high as compared to ent-norsecurinine. They reported *Helminthosporium* species as most resistant to the chemical as *H. gramineum* was not inhibited even at 1,500 ppm. On the contrary, ent-norsecurinine inhibited *H. echinoclova* and *H. penniseti* significantly at 1,000 ppm. Maurya *et al.* (2001) reported complete spore germination inhibition of *Colletotrichum* species, *C. lunata*, *Helminthosporium* species and *Heterospora* at 200, 400, 600, 800, 1,000 ppm of tetrahydropalmatine. A number of chemical compounds isolated from plants have already been reported to be antifungal (Bracher *et al.*, 1994; Mitcher *et al.*, 1975). The present chemical was effective against a varied group of fungi. Its efficacy was significantly high even at low concentrations which suggests the possibility of its use for the control of plant diseases under field conditions. The antifungal property of the present chemical is being reported for the first time.

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