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EFFICACY OF BETAINE HYDROCHLORIDE, A QUATERNARY BASE ISOLATED FROM *PLUCHEA LANCEOLATA*, BASE AGAINST SPORE GERMINATION OF SOME FUNGI

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ABSTRACT

The inhibitory activity of betaine hydrochloride, a quaternary base was evaluated against spore germination of some plant pathogenic fungi. The fungi, *Curvularia lunata*, *Colletotrichum gloeosporioids* and *Heterosporium sp.* were the most sensitive as complete inhibition of germination were observed in all the concentrations (200, 400, 600, 800, 1000 ppm) of the chemical. Similar effects on *Alternaria alternata*, *Curvularia maculans*, *Helminthosporium echinoclova* and *Helminthosporium penniseti* were found at 600, 800, 1000 ppm and *Alternaria brassiae*, *Colletotrichum sp*. and *Fusarium udum* at 800 and 1000 ppm. However, only 1000 ppm was effective against spore germination of *Erysiphe pisi*.

KEYWORDS: *Pluchea lanceolata*, Betaine hydrochloride, Spore germination, Antifungal activity.

INTRODUCTION

Several synthetic fungicides are highly effective in controlling plant diseases but their negative effects on human and animal health and also on the agroecosystem brought about research efforts in developing alternative environmentally acceptable (environment-friendly) methods. Some of the approaches currently pursued biological control, genetic engineering for evolving resistant varieties and the use of induced resistance by biotic and abiotic means

in crop plants.[1] The use of bio-degradable plant products especially from medicinal plants in other aspect gaining importance in plant disease control. Plants contain a wide range of antifungal compounds[2] and many of them particularly phenolics, have been implicated in the natural resistance of several plants against pathogens. Several workers have used crude plant extracts *in vitro* in glass house and under field conditions against several plant pathogens.[3,4] Also various compounds isolated from the plants were shown to be effective against some plant pathogenic fungi *in vitro*[5-9], in glass house[10,11] also in the field.[12,13] Alkaloids are known to affect biological functions of living cells at very low concentration. They often possess an antimicrobial activity.[14,15] The present study was carried out on the betaine hydrochloride, a quaternary base, isolated from *Pluchea lanceolata*.

MATERIALS AND METHODS

The plant *Pluchea lanceolata* Linn (Family: *Asteraecae*) is a small shrub growing wild in the hotter parts of India and is used in Indian system of medicine in various clinical conditions. It is used as a bitter, a laxative, an analgesic, an antipyretic and a nerve tonic, and for treatment of rheumatism, dyspepsia and bronchitis.[16,17] The whole plant was collected from Varanasi district, India. It was identified by Prof. N. K. Dube, Department of Botany and a specimen is kept in the Department. The plant was dried and extracted with methanol by cold percolation. The solvent free extract was extracted with dilute hydrochloric acid. The acidic extract was found to contain very little tertiary bases as tested by Mayer's reagent and paper chromatography, but contained considerable amount of water soluble quaternary bases, which were precipitated by ammonium reineckate. The precipitate obtained was dissolved in acetone, decomposed by adding silver sulphate solution and then converted into base chlorides with barium chloride. The residue obtained on evaporation of the solution to dryness was dissolved in absolute alcohol and the alcohol was evaporated on water bath to dryness. The alcoholic extract was then chromatographed over a column of aluminium oxide. The fractions collected of the alcoholic eluants on crystallization from methanol yielded crystalline needles of hydrochloride, m.p. 242 - 244°C. It was identified as betaine hydrochloride (Fig-1), a quaternary base by comparison with the reported UV, IR and ^{1}HNMR spectral data.[18] It was finally identified by direct comparison with authentic specimen (mixed m.p., co-TLC and superimposable IR).[19] The betaine hydrochloride was thus subjected on spore germination of some plant pathogenic fungi.

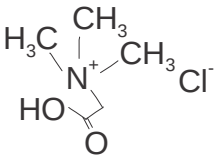

Fig. 1: Structural formula of Betaine hydrochloride.

The test fungi 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 litre). 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 experiments.

Stock solution of betaine hydrochloride (1000 ppm) was prepared by dissolving 5 mg of compound initially in a few drops of methanol in a test tube. After the chemical was completely dissolved, 5 ml of distilled water was added and solvent methanol was evaporated on a water bath. Required concentrations (200, 400, 600, 800, 1000 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 days 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.

RESULTS

The results of the efficacy of betaine hydrochloride against spore germination of some fungi are shown in Table-1.

Table 1: Effect of betaine hydrochloride on spore germination of some fungi.

		Concentration (ppm) Percent spore germination							Critical Difference (CD)
Fugus	**Host**	**C**	**C+M**	**200**	**400**	**600**	**800**	**1000**	
Alternaria alternata	*Saprophyte*	93.26	92.83	65.50	38.35	0	0	0	16.32
A. brassicae	*Brassica campestris*	92.00	90.23	55.50	45.40	13.50**	0	0	14.16
Curvularia lunata	*Oryza sativa*	93.50	92.36	0	0	0	0	0	17.20
C. maculans	*Musa paradisiaca*	94.45	93.15	70.23	45.50	0	0	0	20.23
Colletotrichum sp.	*Arundinaria falcata*	96.06	95.18	68.50	35.50	.16.23**	0	0	18.00
C. glaesporioids	*Mangifera indica*	93.25	91.23	0	0	0	0	0	17.20

Erysiphe pisi	*Pisum sativum*	94.05	93.40	61.23	37.45	8.50**	6.23**	0	19.23
Fusarium udum	*Cajanus cajan*	97.15	96.25	65.32	38.40	3.35**	0	0	16.33
Helminthosporium echinoclova	*Echinoclova species*	96.23	95.45	65.15	30.21**	0	0	0	15.32
H. penniseti	*Pennisetum typhoides*	94.12	92.26	66.58	30.00**	0	0	0	16.11
Heterosporium sp.	*Cassia fistula*	89.23	88.18	0	0	0	0	0	17.15

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

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 solvent methanol was also kept. All experiments were conducted in triplicate.[14]

DISCUSSION

The effect of betaine hydrochloride on spore germination of some plant pathogenic fungi was seen (Table-1). The sensitivity of different fungi to this chemical varied considerably. *Curvularia lunata*, *Colletotrichum gloeosporioids* and *Heterosporium sp.* were the most sensitive as complete inhibition of germination was observed in all the concentrations (200, 400, 600, 800, 1000 ppm) of the chemical. Similar effects on *Alternaria alternata*, *Curvularia maculans*, *Helminthosporoium echinoclova* and *Helminthosporium penniseti* weve recorded at 600, 800 and 1000 ppm, whereas *Alternaria brassiae*, *Colletotrichum sp.* and *Fusarium udum* did not germinate at 800 and 1000 ppm. However, only 1000 ppm was effective against spore germination of *Erysiphe pisi*.

CONCLUSION

A number of alkaloids isolated from plants eg. l-corypalmine[20], narceine methylester and narceine[21], hunnemanine and norsangunarine[22] have already been reported to be antifungal. The present base, betaine hydrochloride was effective against a varied group of fungi. Its efficacy was significantly high even at low concentrations which suggest the possibility of its use for the control of plant diseases under field conditions. The antifungal property of the present base, betaine hydrochloride is being reported here for the first time.

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