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Toxic Spectrum of *Aspergillus niger* Causing Black Mold Rot of Onions

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Abstract: To study the toxic spectrum of *Aspergillus niger*, phytotoxicity, cytotoxicity as well as antimicrobial activity were taken as the test criteria. Culture filtrates of *A. niger* exhibited phytotoxicity against onion and tomato by reducing seed germination and root elongation. The culture filtrates were also tested for cytotoxicity using onion root tip bioassay. Cytological aberrations such as enucleate cells, transfer of chromatin material between adjacent cells and binucleate cells were found in treated onion root tips. Culture filtrates of the fungus grown on yeast extract sucrose broth for one month was extracted with different solvents. Concentrated solvent extracts were tested for antimicrobial activity using disc diffusion method. Butanol and ethyl acetate extracts of the fungal culture filtrates exhibited antimicrobial activity.

Key words: *Aspergillus niger*, phytotoxicity, antimicrobial activity, cytotoxicity

INTRODUCTION

Black mold rot caused by *Aspergillus niger* is often responsible for severe damage to onions during storage. Clusters of black spores of *A. niger* generally form along veins and on or between the outer papery scales of onion bulbs (Rao and Rajasab, 1992).

Infected tissue of onion bulbs initially has a water soaked appearance, later gradually dry and shrivel (Sinclair and Letham, 1996). Black mold caused by *A. niger* in onion can occasionally be seen in the field at harvest, it is a post harvest disease and can cause extensive losses in storage under tropical conditions (Tyson and Fullerton, 2004). Toxigenic molds growing on food raw materials and products made by them, they synthesize and excrete toxins of various chemical compositions that worsen food quality, which becomes hazardous for human and animal health (Lugauskas, 2005). In view of the importance of the mold in the deterioration of onion bulbs and their toxic potential, an attempt was made to study the toxic spectrum of *A. niger* taking phytotoxicity, cytotoxicity and antimicrobial activity as the test criteria.

MATERIALS AND METHODS

Infected onion bulbs were collected from local market of Guntur, A.P. (INDIA) during summer season of 2006 (March- May) and brought to the Microbiology Laboratory of Acharya Nagarjuna University. *A. niger* was isolated from infected onion bulbs and studied for its toxic spectrum. To test the phytotoxic activity, the fungus was cultured on Czapek-Dox broth for five weeks. The culture filtrates collected at weekly intervals under aseptic conditions were tested for phytotoxicity against seed germination and root elongation of onion and tomato as described earlier (Ramamohana Rao and Vijayalakshmi, 2000).

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To test the cytotoxic effects, the fungus was cultured on Czapek-Dox broth for 30 days. At the end of incubation, the culture filtrates were collected under aseptic conditions and then tested for cytotoxic effects against *Allium cepa* bulbs. The culture filtrate without dilution was used as 100% concentration and diluted with sterilized distilled water to attain different concentrations. Triplicated of onion bulbs were maintained for each culture filtrate concentration to study cytotoxicity. The onion bulbs were directly exposed to different concentrations of the culture filtrates such as 100, 50, 25, 10, 5, 1 and 0.9% for 96 h. Root initiation from the onion bulbs as well as the length of roots in treated samples and control was recorded. The root tips cut off from the bulbs were thoroughly washed with distilled water and fixed in absolute alcohol : acetic acid (3:1) for 24 h and then transferred to 70% alcohol. For cytological study, the root tips were hydrolysed in 1 N hydrochloric acid and stained in 2% acetocaramine (Lakshmi and Veeraraghavaiah, 1981). A minimum of 400 cells were observed in order to assess the mitotic manifestations inflicted by the culture filtrate of *A. niger*.

For testing antimicrobial spectrum, the fungus was cultured on yeast extract sucrose broth for one month and screened against gram positive (*Bacillus subtilis* MTCC 441, *B. cereus* MTCC 430 and *Staphylococcus aureus* MTCC 96), gram negative bacteria (*Escherichia coli* MTCC40, *Proteus vulgaris* MTCC 742 and *Pseudomonas aeruginosa* MTCC 424) and yeast (*Candida albicans* MTCC 183). The culture filtrates were extracted with different solvents such as benzene, butanol, chloroform, ethyl acetate, hexane and petroleum ether. Concentrated solvent extracts were employed for testing antimicrobial activity using disc diffusion method (Benson, 1994).

RESULTS AND DISCUSSION

Seed germination and root elongation of onion were adversely affected by the culture filtrates of *A. niger* (Table 1). In onion, reduction in seed germination and root elongation gradually enhanced as the age of the culture advanced. Similar trend was noticed with respect to the seed germination of tomato. But root elongation of tomato was totally suppressed by the culture filtrates collected even from one week old cultures. Toxicity of culture filtrate was persisted after five weeks of cultivation. Root initiation was totally arrested in onion bulbs exposed to different concentrations of culture filtrates except in 0.9%. Analysis of root tips collected from onion bulbs exposed to 0.9% concentration of culture filtrate for mitosis revealed that even at very low concentrations, the culture filtrates of *A. niger* had a toxic mitodepressing effect evidenced by the abnormalities such as cells without nucleus, chromatin transfer and change in the nuclear position as well as number (Fig. 1).

Among the six solvents used for the extraction of secondary metabolites from *A. niger*, butanol and ethyl acetate proved to be good as the solvent extracts were inhibitory to the test organisms (Table 2). *P. vulgaris* appeared to be highly sensitive to the butanol extracts of the culture filtrates followed by *B. cereus* and *S. aureus*. Ethyl acetate extracts of culture filtrates were not as effective as butanol extracts in inhibiting the growth of bacteria and yeast culture. Ali-Siddiqui *et al.* (2001) reported that the compounds in culture filtrate of *A. niger* were thermostable and water soluble fraction was more cytotoxic compared to ethyl acetate and hexane extract.

Table 1: Phytotoxicity of the culture filtrates of *Aspergillus niger*

Incubation period of the culture (days)	Reduction in seed germination (%)		Reduction in root elongation (%)	
	Onion	Tomato	Onion	Tomato
7	29	60	48	100
14	36	70	61	100
21	36	80	64	100
28	57	90	67	100
35	57	100	69	100

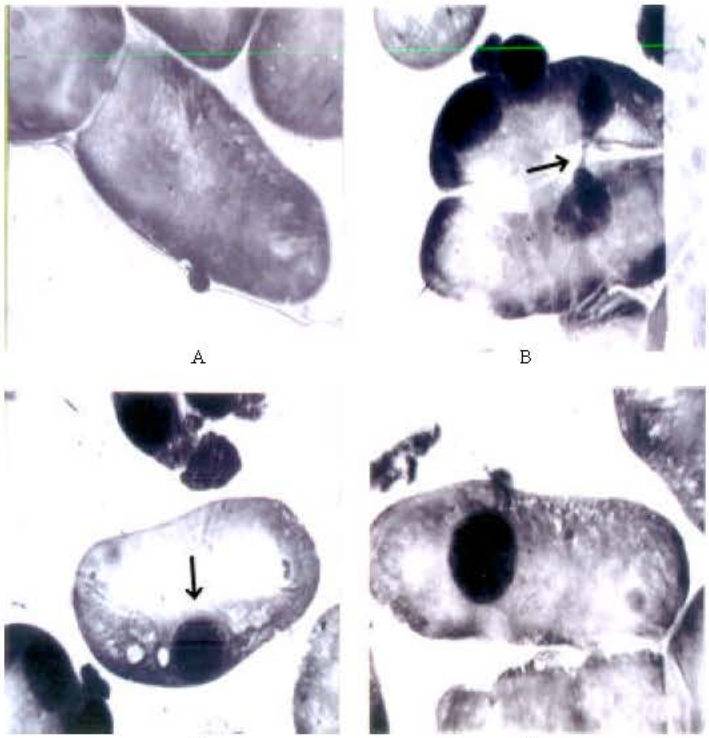

Fig. 1: Cytotoxic effects of *Aspergillus niger* on *Allium cepa* roots, (A) Enucleate cell, (B) Chromatin transfer from one cell to other cell, (C) Displacement of nucleus to a side of the cell and (D) Normal cell

Table 2: Antimicrobial activity of *Aspergillus niger*

	Area of inhibition zone (mm^2)	
Test organism	Butanol extract	Ethyl acetate extract
Bacillus subtilis	8.58	3.30
Bacillus cereus	9.68	2.64
Candida albicans	2.60	-
Escherichia coli	6.50	1.03
Proteus vulgaris	17.60	4.80
Pseudomonas aeruginosa	4.80	1.03
Staphylococcus aureus	9.68	2.64

Phytotoxic effects of *A. niger* have been reported by several workers against wheat (Singh *et al*., 1984), groundnut, sunflower and safflower (Deshpande and Kulkami, 1990) and chilli (Asalmol *et al*., 2001). In the present study culture filtrates of *A. niger* isolated from onion bulbs affected with black mold exhibited high phytotoxicity against onion and tomato, which is evidenced by the inhibition of seed germination and root elongation. Root elongation was more affected by culture filtrate of *A. niger* from onion when compared with seed germination. Zarin *et al*. (2001) also reported that metabolite from *A. niger* were found most successful in minimizing root length. The culture filtrates of *A. niger* were found to inhibit spore germination of fungi such as *Alternaria alternata*, *Curvularia lunata* and *Fusarium roseum* (Kanna, 1979). Kulfinski and Pappelis, (1976) reported that some strains of *A. niger* reduced nuclear dry mass of cells of onion by 11% in comparison with normal cells. The metabolites of *A. niger* from onion exhibited a wide range of toxic spectrum against seed germination, root elongation and microorganisms.

Curtis *et al*. (1974) reported a bioactive compound, Malformin in *Aspergillus niger* infected onion bulbs. The fungal metabolites of *Paecilomyces canescens*, *Aspergillus fumigatus*, *Syncephalastrum racemosum*, *Aspergillus terreus* and *Mucor hiemalis* strongly suppressed cell division and resulted several abnormalities through different mitotic stages (Abdou *et al*., 1989). The mutagenic effects produced by these fungal metabolites reflect the risk that might tale place through the consumption of these contaminated food stuffs. Cytological aberrations in onion root tips treated with the culture filtrates of *A. niger* from onion are reported for the first time. In view of the high toxic spectrum, attempts are in progress to analyse the toxic compounds produced by *A. niger* pathogenic to onions.

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