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PATHOGENIC POTENTIALITY OF FUNGI ASSOCIATED WITH THE SEEDS OF DIFFERENT COTTON (*GOSSYPIUM HIRSUTUM* L.) VARIETIES IN BANGLADESH

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Key words: Cotton seeds, Pathogenic potentiality, Seedling mortality, Vigor index

Abstract

A total of 24 species of fungi, namely *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* (Type 1 and Type 2) Van Tiegh, *A. ochraceus* K. Wilhelm, *A. nidulans* Eidam, *Aspergillus* sp.1, *Aspergillus* sp.2, *Aspergillus* sp.3, *Curvularia lunata* (Wakker) Boedijn, *Colletotrichum gloeosporioides* Penz & Sacc, *C. gossypii* Southw., *Chaetomium globosum* Kunze., *Fusarium nivale* (Fr.) Sorauer, *F. moniliforme* J. Shelden, *F. oxysporum* Schlechtendal, *F. fujikuroi* Nirenberg, *Mucor* sp. P. Micheli ex L., *Penicillium* sp.1 and sp.2 Link, *Rhizoctonia solani* Khun., *Rhizopus stolonifer* (Ehrenb.) Vuill., *Rhizomucor* sp. Lucet & Costantin, *Syncephalastrum racemosum* Cohn and *Trichoderma viride* Pers. were found to be associated with the seeds of 14 varieties (CB 1- CB 14) of cotton. Out of these 24 fungal species, nine were found to be pathogenic to cotton. They were *Aspergillus flavus, A. niger* (Type 1), *Aspergillus* sp. 1, *Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and *Rhizoctonia solani.* These pathogenic fungi had remarkable effect on seed germination, vigor index, root-shoot length and mortality of cotton seedlings. The germination percentage of control seeds was 88 but because of the presence of pathogenic fungi the rate showed considerable reduction in all the varieties and it varied from 20 to 82%. Among the nine fungal isolates *Rhizoctonia solani* showed maximum reduction in seed germination. *Aspergillus flavus, Colletotrichum gloeosporioides* and *Fusarium moniliforme* also caused near about 50% reduction in seed germination. Mortality percentage of control seedling were also less (6) whereas, in inoculated seeds it was higher and varied from 7 - 23%. Root- shoot ratio of control seedlings was high but less in inoculated seedlings. The vigor index of control plant was high (1548.8) but less in inoculated plants. The lowest vigor index was noticed for *Rhizoctonia solani* (202.0) and highest for *Aspergillus* sp. 1(1213.6). Results indicated that *Curvularia lunata* and *Rhizoctonia solani* showed a greater impact in reduction of cotton seed germination and vigor index.

Introduction

Cotton (*Gossypium* spp.) is the major textile fiber with high commercial value in the world and playing a key role in the economic and social welfare[1]. In Bangladesh, till now 14 upland cotton (*Gossypium hirsutum* L.) varieties (CB-1 to CB-14) have been released. Among these varieties CB-12, CB-13 and CB-14 are high yielding with high GOT (Ginning outturn) and good fiber characteristics[2]. Cotton is the second important

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cash crop in Bangladesh after Jute. It is grown primarily as a fiber crop, but after the lint, the seed can be crushed to extract vegetable oil and protein rich animal food[3]. The cotton is directly connected to most essential needs of people of the world among all other crops. It is cultivated in more than 70 countries, which represents 2.5% of the all cultivated land and grows mostly in tropical and subtropical regions of the world.

Fungi are the largest group of the seed-borne pathogens and most of the seed transmitted pathogens are fungi. Seed-borne fungi are a serious problem worldwide causing diseases and poor quality of many crops. *Alternaria*, *Fusarium, Macrophomina*, *Rhizoctonia* and several other fungi were frequently isolated from cotton seeds and seedlings[4]. *Rhizoctonia solani, Pythium ultimum, Fusarium oxysporum*, *F. moniliforme, F. semitectum* and several other fungi were isolated from cotton seeds and most of them were found to be pathogenic to cotton[5].

Alternaria alternata, Aspergillus niger, Fusarium acuminatum, Fusarium solani, Pythium ultimum, Rhizopus arrhizus and *Rhizoctonia solani* were isolated from cotton seeds by Mansoori and Hamdolahzadeh[6]. A number of seed-borne pathogenic fungi such as *Alternaria* spp., *Fusarium* spp., *Rhizopus* spp. and *Aspergillus* spp. are the most frequently identified in cotton seeds[7]. Both Egyptian and American cotton varieties were susceptible at different degrees to *R. solani* [8].

Seed-borne pathogenic fungi are a continuing problem and may even be responsible for the re-emergence of diseases of the past as well as the introduction of diseases into new areas. They may result in loss in germination, discoloration and shriveling, development of plant diseases, distribution of pathogen to new areas, introduction of new strains or physiologic races of the pathogen along with new germplasm from other countries and toxin production in infected seed[9]. Fungi attack plants and cause a very serious economic impact on agricultural production due to their ability to induce diseases of cultivated crops that result in important yield losses[10].

The study of seed-borne pathogens is necessary to determine seed health and to improve germination potential of seed which finally leads to increase of the crop production. Seed health testing to detect seed-borne pathogens is an important step in the management of crop diseases [11].

Pathogens associated with seeds cause germination failure, post emergence seedling infection and also seedling blight. A very little information is available in our country about seed processing and seed-borne pathogenic fungi of upland cotton. So the present work was undertaken to search the pathogenic fungi associated with different varieties of upland cotton seeds. The objective of this study was to evaluate the effect of pathogenic fungi on the germination, seedling mortality, vigor index and seedling root shoot length.

Materials and Methods

Seed samples of CB1-14 were collected from Cotton Research, Training and Seed multiplication Farm, Gazipur after harvesting and kept in clean glass jars, labeled properly and preserved at room temperature for subsequent use. The experiment was conducted in the Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka.

The fungi were isolated from the samples following the Tissue Planting method on PDA medium[12], Blotter method and Paper Towel method[13]. Three hundred seeds of each samples were used for all the methods. Isolated fungi were transferred to separate PDA plates and PDA slants for further studies and preservation.

Identification of the isolates were determined on the basis of morphological characteristics observed under a compound microscope following the standard literatures[14-22]. Per cent frequency of the occurrence of the fungal isolates was calculated by adopting the following formula of Spurr and Wetly[23].

For determination of germination, 300 surface sterilized seeds of each sample were taken and placed in 30 PDA plates. Plates were then incubated at room temperature for 7 days. Seeds producing both plumule and radical were considered as germinated seeds. Germination was recorded after 7 days and expressed as percentage.

The seed quality, seedling mortality and seedling height of different varieties of cotton seeds were determined according to the method described by Shamsi and Khatun[24]. The seedling vigor index of different cotton varieties was recorded with the formula of Lee *et al*.[25].

Pathogenicity test of the isolated fungi was done following seed inoculation technique[26]. Two hundred seeds of each variety were selected and soaked in distilled water in the beaker for 30 minutes separately and then surface sterilized with 10% Chlorox for 5 minutes. Spore suspension of the test fungi at 10^4 /ml concentration was prepared in a 250 ml sterilized beaker. Three hundred seeds from each variety were placed in beakers with spore suspension and then left undisturbed for 2 hours. One hundred of each healthy, spotted and inoculated seeds of each cotton varieties were selected and single seed was placed in sterilized 8 inch cotton plugged test tubes containing 10 ml 2% water agar medium. Healthy seeds served as control. Observation was made for 3 weeks at 3 days intervals. Germination percentage of seeds, development of disease symptoms, seedling mortality and root-shoot length of seedling were recorded on healthy and inoculated seeds of each cotton varieties.

After 15 days of inoculation, pathogenic fungi were re-isolated from diseased and inoculated cotton seeds and confirmed their identity following Koch's postulates where healthy seeds and seedlings remained fresh.

Data were evaluated by analysis of variance (ANOVA) by using STAR statistical program and means were compared using Duncan's Multiple Range Test (DMRT).

Results and Discussion

A total of 24 species of fungi *viz*., *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* (Type 1 and Type 2) Van Tiegh, *A. ochraceus* K. Wilhelm, *A. nidulans* Eidam, *Aspergillus* sp. 1, *Aspergillus* sp. 2, *Aspergillus* sp. 3, *Curvularia lunata* (Wakker) Boedijn, *Colletotrichum gloeosporioides* Penz & Sacc, *C. gossypii* Southw., *Chaetomium globosum* Kunze., *Fusarium nivale* (Fr.) Sorauer, *F. moniliforme* J. Shelden, *F. oxysporum* Schlechtendal, *F. fujikuroi* Nirenberg, *Mucor* sp. P. Micheli ex L., *Penicillium* sp.1 and sp.2 Link, *Rhizoctonia solani* Khun., *Rhizopus stolonifer* (Ehrenb.) Vuill., *Rhizomucor* sp. Lucet & Costantin, *Syncephalastrum racemosum* Cohn and *Trichoderma viride* Pers. were isolated and identified from the seeds of 14 varieties (CB 1- CB 14) of cotton. All the isolated fungi were selected for pathogenicity test.

Out of 24 isolated fungi, nine showed positive results during pathogenicity test. They were - *Aspergillus flavus*, *A. niger* (Type 1), *Aspergillus* sp. 1, *Colletotrichum gloeosporioides, Curvularia lunata, Fusarium nivale, F. moniliforme, Mucor* sp. and *Rhizoctonia solani* (Figs 1,2).

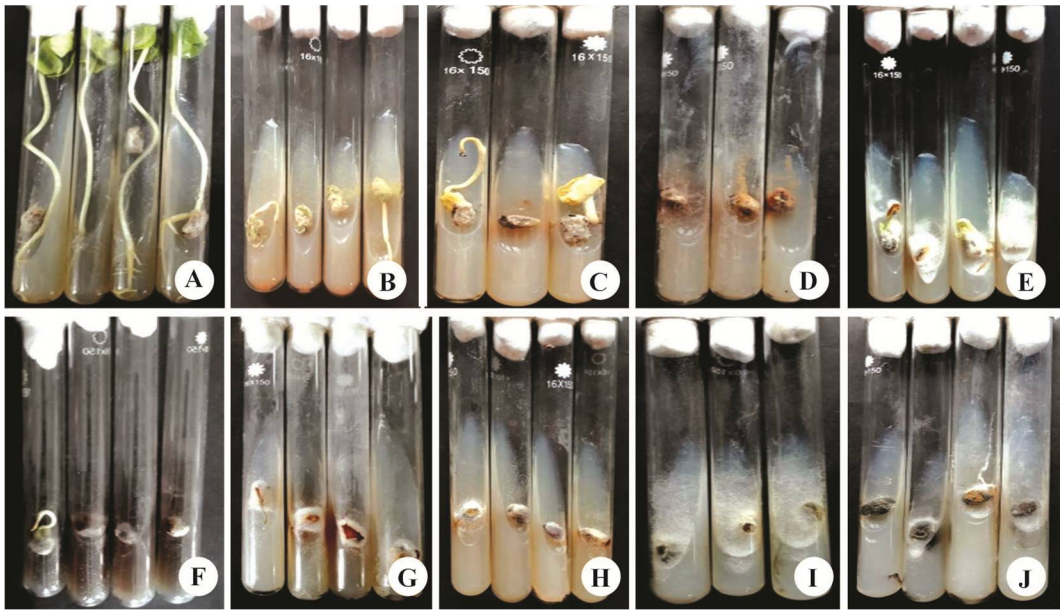

Fig. 1. Pathogenecity test of the isolated fungi. A. Control, B-J. Inoculated seeds: B. *Aspergillus flavus,* C. *Aspergillus niger* (Type 1), D. *Aspergillus* sp. 1, E. *Colletotrichum gloeosporioides,* F. *Curvularia lunata*, G. *Fusarium nivale,* H. *Fusarium moniliforme*, I. *Mucor* sp. and J. *Rhizoctonia solani*.

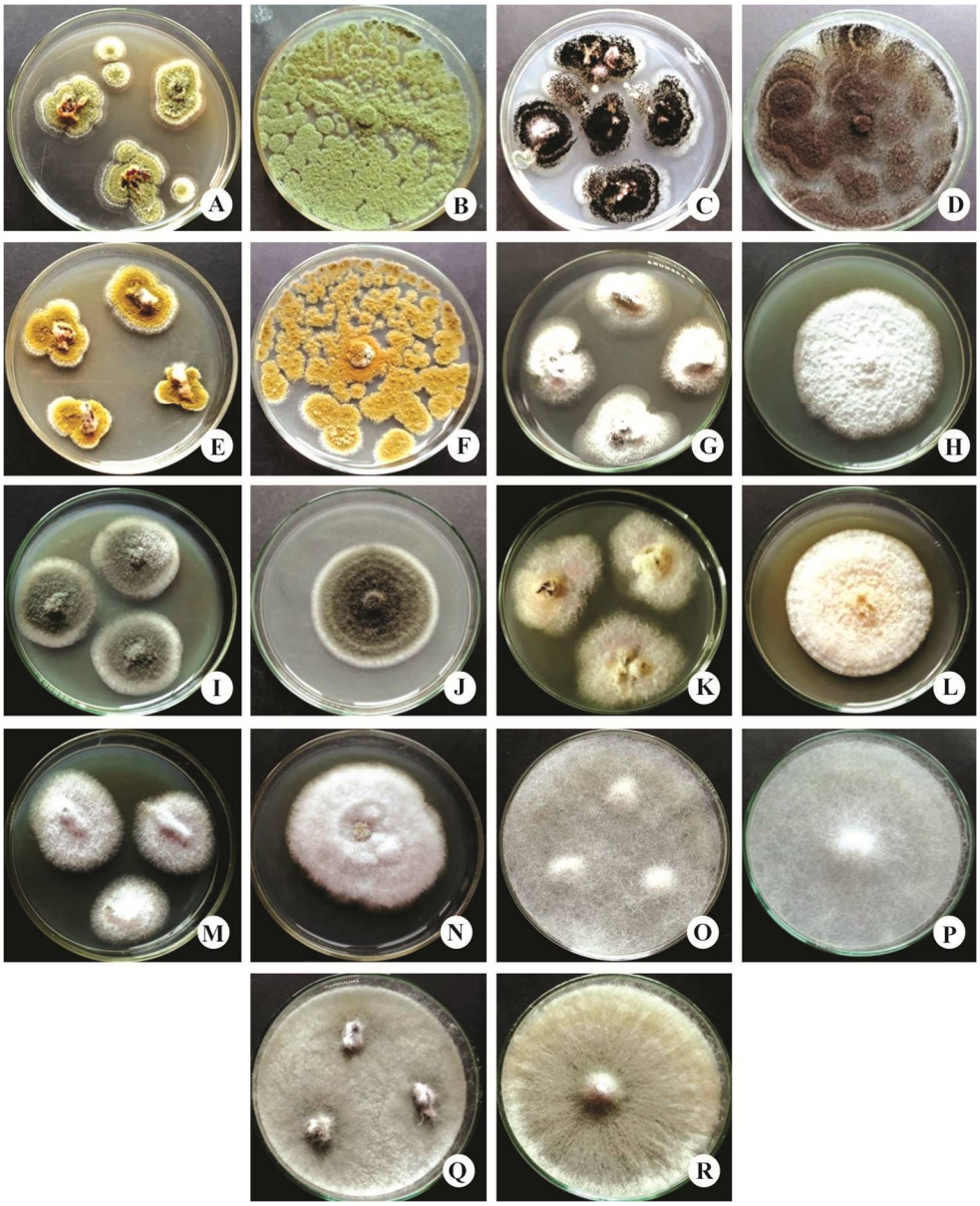

Fig. 2. Re-isolated colony and pure culture plates of the pathogenic fungi. A-B. *Aspergillus flavus,* C-D. *Aspergillus niger* (Type 1), E-F. *Aspergillus* sp. 1, G-H. *Colletotrichum gloeosporioides,* I-J. *Curvularia lunata*, K-L. *Fusarium nivale,* M-N. *Fusarium moniliforme*, O-P. *Mucor* sp. and Q-R. *Rhizoctonia solani*.

The effect of test fungi on the seeds of cotton are presented in Table 1. Present study revealed that, the germination percentage of control seeds was 88 in all varieties but because of the presence of pathogenic fungi, the germination rate showed a considerable reduction in the inoculated seeds and it varied from 20 - 85%. Among the nine

pathogenic fungi, *Rhizoctonia solani* showed maximum reduction in cotton seed germination (20%). *Aspergillus flavus, Colletotrichum gloeosporioides* and *Fusarium moniliforme* also caused near about 50% reduction in seed germination (Table 1 and Fig. 1).

Table 1. Effect of pathogenic fungi on seed germination, seedling mortality, root-shoot length and vigor index in different varieties of cotton seeds.

Treatments	Germination (%)	Mortality (%)	Root-shoot length (cm)		Vigor index
			Root	Shoot	
Control seeds	88^{a}	6^{f}	6.8^{a}	10.8^{a}	1548.8^{a}
Inoculated seeds					
Aspergillus flavus	40^{g}	7^{ef}	2.7^{g}	4.8^{i}	300.0^{h}
Aspergillus niger (Type-1)	73^{c}	20^{b}	4.1^{d}	8.0^{f}	883.3^{e}
Aspergillus sp. 1	82^{b}	11^{d}	5.2^{b}	9.5^{d}	1213.6^{b}
Colletotrichum gloeosporioides	40^{g}	9de	4.2^{d}	8.1^{f}	488.0^{g}
Curvularia lunata	21^{h}	20^{b}	3.7^{e}	8.7^{e}	260.4^{i}
Fusarium moniliforme	47^{f}	14^{c}	3.6^{e}	7.4^{g}	517.0^{f}
Fusarium nivale	69^{d}	22^{ab}	5.3^{b}	10.3^{b}	1076.4^{c}
Mucor sp.	60^{e}	23^{a}	4.8^{c}	10.0^{c}	888.0^{d}
Rhizoctonia solani	20^{h}	20^{b}	3.0^{f}	7.1^{h}	202.0^{j}
CV%	1.85	6.58	2.30	1.18	0.09

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.

Mortality percentage of control seeds was also less (6) whereas, in inoculated seeds it was higher and varied from 7 - 23. The highest mortality percentage was 23.0 in *Mucor* sp. inoculated seeds and the lowest mortality percentage was 7.0% in *Aspergillus flavus* inoculated seeds. Root-shoot ratio of control seeds was high but less in inoculated seeds. In healthy seeds, the average shoot length was 10.8 cm whereas the highest shoot length 10.3 cm was recorded on *Fusarium nivale* inoculated seeds and lowest shoot length 4.8 cm was recorded on *Aspergillus flavus* inoculated seeds. In healthy seeds, the average root length was 6.8 cm whereas the highest root length 5.3 cm was observed in *Fusarium nivale* inoculated seeds and lowest root length 2.7 cm was shown by *Aspergillus flavus* inoculated seeds (Table 1 and Fig. 3).

The vigor index of control plant was 1548.8 but more or less in inoculated plants. The lowest vigor index was noticed for *Rhizoctonia solani* (202.0) and highest for *Aspergillus* sp. 1 (1213.6) (Table 1). Among the isolated fungal pathogens, *Curvularia lunata* and *Rhizoctonia solani* showed a greater impact in reduction of cotton seed germination and reduced vigor index.

Aspergillus flavus, *A. niger* (Type-I), *Curvularia lunata, Fusarium moniliforme* var. *subglutinans*, *F. sporotrichioides* and *Rhizoctonia solani* were found to be pathogenic for 3 hill cotton (*Gossypium arboreum*) varieties in Bangladesh by Naznin and Shamsi[27].

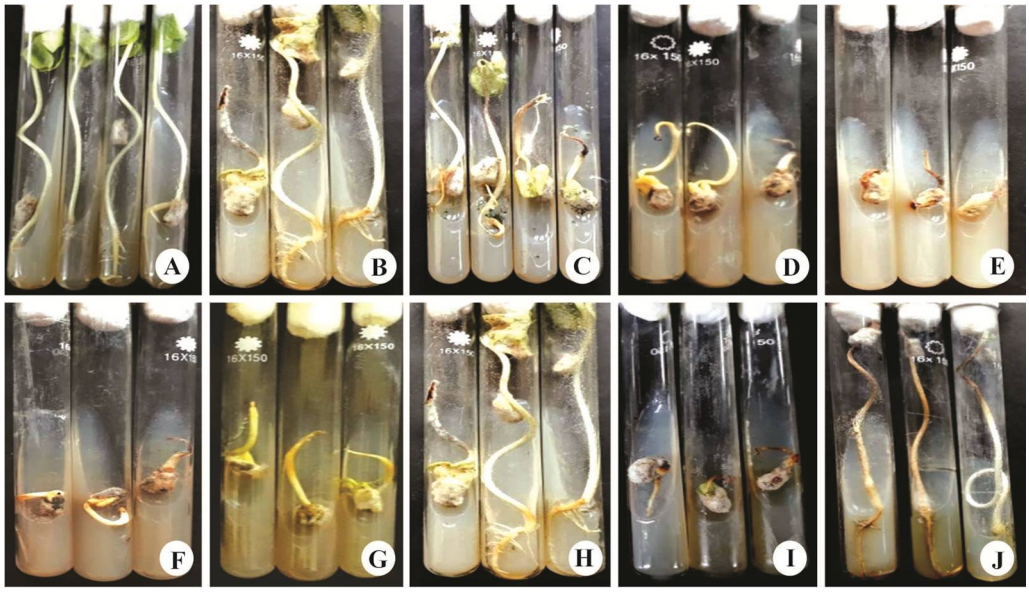

Fig. 3. Mortality of the cotton seedlings due to the presence of pathogenic fungi. A. Control, B-J. Inoculated seedlings: B. *Aspergillus flavus,* C. *Aspergillus niger* (Type 1), D. *Aspergillus* sp. 1, E. *Colletotrichum gloeosporioides,* F. *Curvularia lunata*, G. *Fusarium nivale,* H. *Fusarium moniliforme*, I. *Mucor* sp. and J. *Rhizoctonia solani*.

Palmateer *et al.*[28] found that *Fusarium moniliforme, F. semitectum* and *F. solani* were the most pathogenic fungi causing mortality of cotton plants.

Rhizoctonia solani is the most important pathogen involved in cotton seedling disease in Egypt[29]. Pre- or post-emergence cotton seedling damping-off, caused by *R. solani,* can be quite serious in the United States and often results in a substantial stand loss[30].

Wang *et al.*[31] isolated F*. moniliforme, F. semitectum, F. oxysporum, F. solani, F. equiseti* and *F. compactum* from cotton seedlings and bolls and they found that *Fusarium moniliforme* was the predominant pathogen causing seedling and boll red rot of cotton.

From the above result it can be concluded that all the isolates are potential phytopathogens which can lead to severe crop damage and they showed remarkable effect on seed germination, seedling mortality, vigor and root-shoot height. This result will be useful for designing control measure of seed-borne fungi and production of healthy seeds of cotton.

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