

PATHOGENIC POTENTIALITY OF FUNGI ISOLATED FROM SEEDS OF THREE HILL COTTON VARIETIES (*GOSSYPIUM ARBOREUM* L.)

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Abstract

Twelve species of fungi, namely *Aspergillus flavus* Link., *A. fumigatus* Fresenius., *A. niger* van Tiegh (Type-I), *A. niger* van Tiegh (Type-II), *Chaetomium globosum* Kunze ex Fr., *Curvularia lunata* (Wakker) Boedijn, *Fusarium moniliforme* var. *subglutinans* Wr. & Reink, *F. sporotrichioides* Sherb., Mem., *Penicillium* Link., *Pestalotiopsis guepinii* (Desm.) Stay., *Rhizoctonia solani* J.G. Kühn, *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill and *Trichoderma viride* Pers were isolated from the seeds of three cotton varieties (HC-1, HC-2 and HC-3) following "Tissue planting" and "Blotter" methods. Among the isolated fungi, six, namely *A. flavus*, *A. niger*, *C. lunata*, *F. moniliforme* var. *subglutinans*, *F. sporotrichioides* and *Rhizoctonia solani* showed pathogenic potentiality following seed inoculation technique. These pathogenic fungi had remarkable effect on seed germination, root shoot length and mortality of cotton seedlings.

Introduction

The genus *Gossypium* comprises around 50 species⁽¹⁾ of which *Gossypium arboreum* is grown in hilly regions of Bangladesh mainly Chittagong and the Chittagong hill tracts. Economically it is very important as the lint is of superior quality, its staple is coarse and very short but very strong⁽²⁾. In Bangladesh three hill cotton varieties (HC-1, HC-2 and HC-3) have been cultivated. It is locally called Comilla cotton or Hill cotton.

Every year the yield of cotton production is decreased by different seedling disease which mainly caused by fungi, bacteria and viruses. These organisms often cause stunting of the plants, defoliation, reduced vigor and yield and sometimes death^(3,4). The most common type of diseases that are seen in cotton are seedling blight, boll rot, leaf spot and leaf blight, black root rot etc. Most deteriorating pathogens associated with cotton boll rot are *Rhizoctonia* spp., *Aspergillus* spp., *Fusarium* spp., *Alternaria* spp., *Diplodia* spp., *Sclerotium* spp., *Rhizopus* spp. and several other fungi^(5,6). Leaf spot and leaf blight caused by *Cercospora* spp., *Alternaria* spp., *Cochliobolus* spp., *Bipolaris* spp., *Myrothecium* spp., *Curvularia* spp., *Rhizoctonia* spp. and *Stemphylium* spp.^(7,8). Leaf blights may happen at any stage of plant growth. Seedling diseases are generally caused by

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Thielaviopsis spp., *Rhizopus* spp., *Rhizoctonia* spp., *Pythium* spp., *Alternaria* spp., *Aspergillus* spp., *Pythium* spp. and *Fusarium* spp.⁽⁹⁻¹¹⁾ Boll rot of cotton caused by *Sclerotium rolfsii* Sacc. and *Rhizopus oryzae* Went & Prins. Geerl. was first time recorded from Bangladesh.⁽¹²⁻¹³⁾ Lutfunnessa and Shamsi (2011) reported diseases of four varieties of cotton plant.⁽¹⁴⁾ Shamsi *et al.* (2015) reported three Deuteromycetous fungi on cotton plant from Bangladesh.⁽¹⁵⁾

There is so far no record on seed borne diseases of Hill cotton in Bangladesh. Therefore, present investigation was undertaken to determine the pathogenic potentiality of the fungi associated with *G. arboreum* seeds.

Materials and Methods

Seeds of three varieties of cotton (*Gossypium arboreum*), namely HC-1, HC-2 and HC-3 were collected from Cotton Development Board (CDB), Khamarbari, Farmgate, Dhaka. Quality status of three varieties of cotton seeds were determined by seed quality analysis. Further, purity percentage of seeds was determined with the following formula:

$$\text{Purity percentage of seed} = \frac{\text{Weight of pure seed}}{\text{Total weight of seed}} \times 100$$

Fungi associated with cotton seeds were isolated following 'Blotter' and 'Tissue Planting' method⁽¹⁶⁾. Identification of the isolated fungi was determined following standard literatures⁽¹⁷⁻²³⁾.

Pathogenicity test of isolated fungi was made following seed inoculation technique⁽²⁴⁾. Six hundred seeds were selected from each variety of cotton seeds and soaked in distilled water in three beakers for 30 minutes separately and then surface was sterilized with 10% Clorox for five minutes. Spore suspension of the test fungus at 10⁴ /ml concentration was prepared in a 500 ml sterilized beaker. Three hundred seeds from each variety were placed in 250 ml beakers. Hundred ml of spore suspension with individual spore were added in seeds of each beaker and left undisturbed for 2 hours. Three hundred of each healthy and inoculated seeds of three cotton varieties were selected and single seed was placed in sterilized 6 inch cotton plugged test tubes containing 10 ml (2% agar) water agar medium. Healthy seeds served as control. Observation was made for 2 weeks at 3 days interval. Germination percentage of seeds, seed mortality and root shoot length of seedlings were recorded on healthy and inoculated seeds of three cotton varieties.

The pathogens were re-isolated from the inoculated cotton seeds and confirmed their identity following Koch's postulates.

Data were evaluated by ANOVA by using STAR statistical program and means were compared using DMRT.

Results and Discussion

For seed quality analysis the percentage of pure seeds, abnormal seeds and inert matter is presented in Table 1. Seed quality analysis showed that the percentage of pure seeds of HC-1, HC-2 and HC-3 cotton varieties were 97, 96 and 97%, respectively. The highest per cent of the inert matter (1.26) was found in HC-2 variety and the lowest (0.86) in HC-1 variety. The highest per cent of abnormal seeds (2.74) was recorded in HC-2 whereas the lowest count (2.04) was recorded in HC-1 (Table 1).

Table 1. Purity status of the cotton seeds collected from CDB, Khamarbari, Farmgate, Dhaka.

Cotton varieties	Pure seeds (% weight)	Abnormal seeds (% weight)	Inert matter (% weight)
HC-1	97	2.04	0.96
HC-2	96	2.74	1.26
HC-3	97	2.14	0.86

A total of 12 species of fungi viz., *Aspergillus flavus*, *A. fumigatus*, *A. niger* (Type-I), *A. niger* (Type-II), *Chaetomium globosum*, *C. alunata*, *F. moniliforme* var. *subglutinans*, *F. sporotrichioides*, *Penicillium* sp., *Pestalotiopsis guepinii*, *Rhizoctonia solani*, *Rhizopus stolonifer* and *Trichoderma viride* were isolated and identified from the seeds of three variety of cotton. All the isolated fungi were selected for pathogenicity test.

Out of 12 isolated fungi, a total of six fungi showed positive results during pathogenicity test. They were *A. flavus*, *A. niger* (Type-I), *C. lunata*, *F. moniliforme* var. *subglutinans*, *F. sporotrichioides* and *Rhizoctonia solani* (Figs 1 and 2).

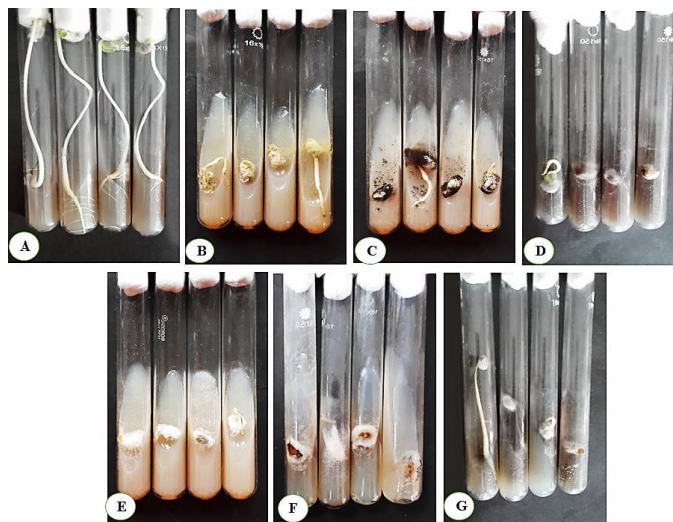


Fig. 1. Pathogenicity test of six isolated fungi. A. Control (Uninoculated seeds); B-G. Inoculated seeds: B. *Aspergillus flavus*, C. *Aspergillus niger* (Type-I), D. *Curvularia lunata*, E. *Fusarium moniliforme* var. *subglutinans*, F. *F. sporotrichioides* and G. *Rhizoctonia solani*.

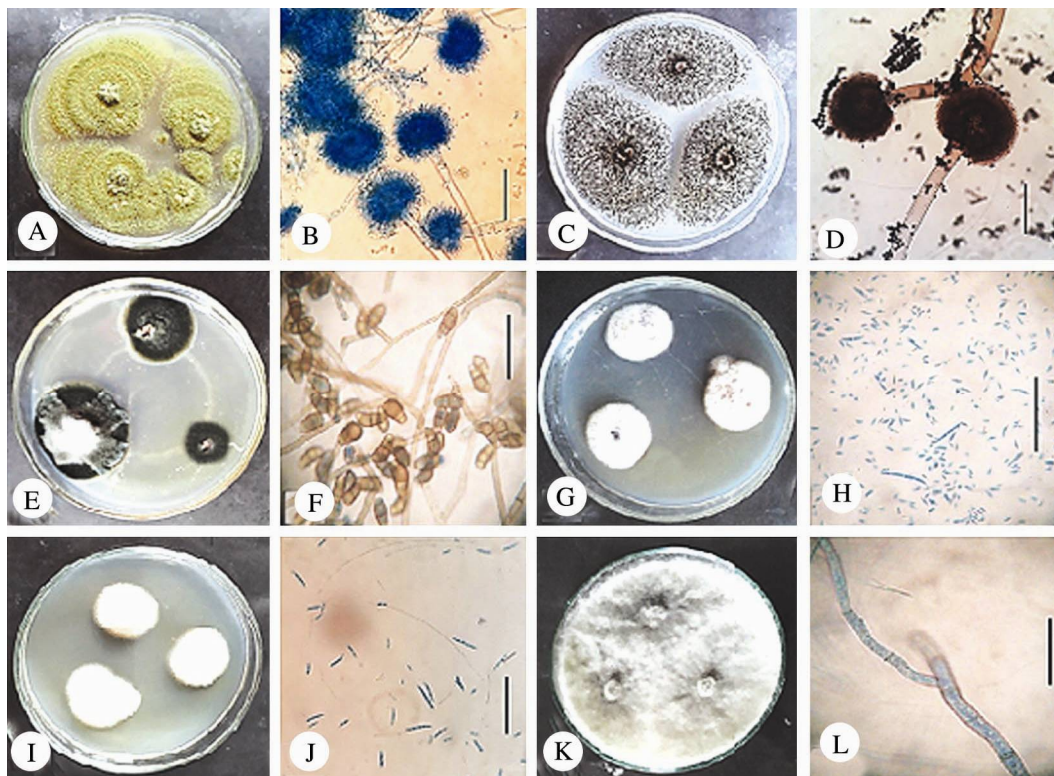


Fig. 2. Re-isolated fungal colony and microscopic images of pathogens. A-B. *Aspergillus flavus*, C-D. *Aspergillus niger* (Type-I), E-F. *Curvularia lunata*, G-H. *Fusarium moniliforme* var. *subglutinans*, I-J. *F. sporotrichioides* and K-L. *Rhizoctonia solani*.

The effect of test fungi on the seeds of cotton are presented in Table 2 and Fig. 3. *Aspergillus flavus*, *A. niger* (Type-I), *Curvularia lunata*, *Fusarium moniliforme* var. *subglutinans*, *F. sporotrichioides* and *Rhizoctonia solani* reduce the length of roots and shoots of cotton seedling. In uninoculated healthy seeds, the average shoot length was 85 mm whereas the highest shoot length 53.6 mm was recorded on *A. niger* (Type-I) inoculated seeds and lowest shoot length 34.5 mm was recorded on *A. flavus* inoculated seeds (Table 2 and Fig. 3). In healthy seeds, the average root length was 48.4 mm whereas the highest root length 37.0 mm was observed in *C. lunata* inoculated seeds and lowest root length 20.4 mm was shown by *F. sporotrichioides* inoculated seeds (Table 2 and Fig. 3). Healthy seeds showed 100% germination whereas the highest germination percentage was 70 in *A. niger* (Type-I) inoculated seeds and the lowest germination percentage was 55 in *F. sporotrichioides* inoculated seeds (Table 2, Fig. 3). No inoculated / healthy seeds showed 3.00% seedling mortality whereas the highest mortality percentage was 57 in *F. sporotrichioides* inoculated seeds and the lowest mortality percentage was 28.6 in *A. niger* inoculated seeds (Table 2, Fig. 3).

Table 2. Effects of pathogenic fungi on different parameters of cotton seeds (*Gossypium arboreum* L.).

Uninoculated and inoculated seeds	Average shoot length (mm)	Average root length (mm)	Germination (%)	Mortality (%)
Uninoculated seeds	85.00 ^a	48.40 ^a	100 ^a	3.00 ^e
Inoculated seeds				
<i>Aspergillus flavus</i>	34.50 ^e	20.83 ^d	60.00 ^{cd}	50.00 ^a
<i>A. niger</i> (Type-I)	53.63 ^b	30.00 ^c	70.00 ^b	28.57 ^d
<i>Curvularia lunata</i>	48.00 ^c	37.00 ^b	60.00 ^{cd}	31.00 ^{cd}
<i>Fusarium moniliform</i> var. <i>subglutinans</i>	46.00 ^c	29.57 ^c	60.00 ^{cd}	33.30 ^c
<i>F. sporotrichioides</i>	40.00 ^d	20.43 ^d	55.00 ^d	38.00 ^b
<i>Rhizoctonia solani</i>	46.53 ^c	23.47 ^d	65.00 ^{bc}	50.00 ^a
CV%	1.85	4.03	2.76	3.96

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

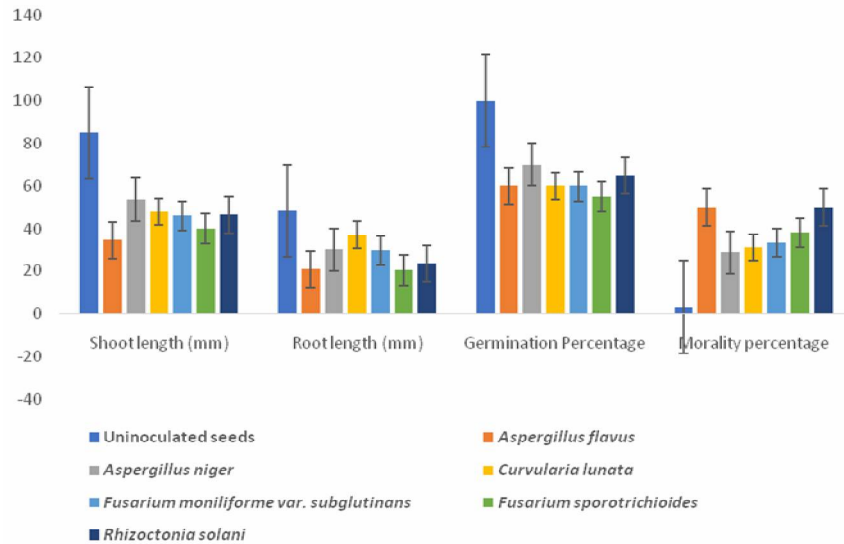


Fig. 3. Effects of pathogenic fungi on different parameters of cotton seeds (*Gossypium arboreum* L.).

Kumar *et al.*⁽²⁵⁾ reported that *Aspergillus* sp. and *Fusarium* sp. reduced the seed germination by causing sesame seed rot. From the above results, it is clear that the six test fungi have remarkable effect on seed germination, root shoot length and mortality of seedlings.

This results will be useful for designing control measure of seed borne fungi and production of healthy seeds of cotton.

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