

Original Article

**Prevalence of fungi associated with seeds of three cotton varieties (*Gossypium arboreum* L.) in storage**

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**ABSTRACT:** Twelve species of fungi were isolated from the seeds of three cotton varieties (HC-1, HC-2 and HC-3) following “Tissue planting” and “Blotter” methods. The isolated fungi were *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* van Tieghem (Type-I), *A. niger* van Tieghem (Type-II), *Chaetomium globosum* Kunze ex Fr., *Curvularia lunata* (Wakker) Boedijn, *Fusarium moniliforme* var. *subglutinans* Wr. & Reink, *Fusarium sporotrichioides* Sherb., Mem., *Penicillium* Link, *Pestalotiopsis guepinii* (Desm.) Stay., *Rhizoctonia solani* J.G. Kuhn, *Rhizopus stolonifer* (Ehrenb.: Fr.) Vuill. and *Trichoderma viride* Pers. Association of fungi with cotton seeds was recorded at 30, 90 and 120 days of storage. The fungal associations were varied with duration of storage periods. *Aspergillus niger* (Type-I), *A. niger* (Type-II) and *Rhizoctonia solani* were predominant fungi. *Fusarium sporotrichioides* is a new record for Bangladesh.

**Keywords:** Prevalence, Fungi, Cotton seeds, Storage, Bangladesh

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**INTRODUCTION**

Cotton (*Gossypium* spp.) is one of the most renowned, reliable fiber yielding crops and belongs to the family Malvaceae<sup>1</sup>. The genus *Gossypium* comprises around 50 species of which *G. hirsutum*, *G. barbadense*, *G. arboreum* and *G. herbaceum* are cultivated commercially throughout the world<sup>2</sup>. Among these, *Gossypium arboreum* is grown in hilly regions of Bangladesh and locally known as Hill cotton. Three varieties of hill cotton (HC-1, HC-2 and HC-3) have been cultivated in Bangladesh. In the Chittagong hill tracts, the hilly people cultivate Hill cotton as “Jhum” cultivation.

Cotton plays the major role in economic and social welfare of the world. More than 80 countries in the world and 2.5% cultivable lands are being used to cultivate the cottons. According to FAO (2017), world

cotton production is projected to grow 1.8% annually over the next ten years, reaching 26.1 megatons (Mt) in 2026, which is 12% higher than during the base period.

Every year the yield of cotton production is decreased by different seedling diseases which are mainly caused by fungi, bacteria and viruses. These organisms often cause stunting of the plants, defoliation, reduced vigor and yield and sometimes death<sup>3</sup>. Fungi associated with seeds cause germination failure, post emergence seedling infection and seedling blight. So far 20 types of symptoms and 52 species of fungi have been reported on cotton plant from various cotton growing areas of the world. *Rhizopus* spp., *Fusarium* spp., *Pythium* spp. and *Colletotrichum* spp. are the most commonly associated fungi with cotton diseases<sup>4</sup>.

Most of the dangerous diseases in cotton are transmitted through seed<sup>5</sup>. Seedling diseases are generally caused by *Thielaviopsis* spp., *Rhizoctonia* spp., *Pythium* spp. and *Fusarium* spp.<sup>6,7</sup>.

These fungal species can colonize the seed within hours of planting and can lead to the following effects- (a) Pre-emergence damping off (b) Post-emergence damping off (c) Seed decay before germination and (d) Generalized blight<sup>8,9</sup>.

Seeds in storage carry a mycoflora of 'field' and 'storage' fungi. Field fungi gradually disappear and storage fungi then predominate. Most of the storage pathogens such as *Penicillium* spp., *Aspergillus* spp. and *Rhizopus* spp. are responsible for discoloration of the seeds. These fungi also grow vigorously and initiate grain spoilage. These also bring about several undesirable changes making them unfit for consumption and sowing<sup>10</sup>.

Lot of research has been done at home and abroad on fungal infection of cotton but information on storage mycoflora of cotton seeds is inadequate in Bangladesh<sup>11-14</sup>. Considering the importance of cotton crop, the present research work was undertaken to find out the prevalence of fungi in the seeds of selected cotton (*Gossypium arboreum*) varieties (HC-1, HC-2 and HC-3) in different storage conditions.

## MATERIALS AND METHODS

### Collection of Seed Samples

Seeds of three cotton (*Gossypium arboreum*) varieties namely HC-1, HC-2 and HC-3 were collected from Cotton Development Board (CDB), Khamarbari, Farmgate, Dhaka. Samples were collected during the period of April 2017. The collected seed samples were kept in polythene bag with airtight container in two available storage temperatures viz., room temperature (25±2°C) and 4°C in refrigerator for subsequent use. Experiment was conducted in the Laboratory of Mycology and Plant Pathology, Department of Botany, University of Dhaka.

### Isolation and purification of fungi

Fungi associated with the seeds of cotton varieties were isolated separately following Tissue planting and Blotter method<sup>15-16</sup>. For these methods of isolation approximately 300 seeds with three replications were used for each sample. The seeds were washed with sterile water and then surface sterilized by dipping in 10% chlorox solution for five minutes. The seeds were

again washed with sterile water for three times. Finally, the inocula were placed on the sterile filter paper to remove the excess surface water and kept in room condition.

In Tissue planting method surface sterilized seeds as mentioned above were placed on sterilized Petri plates containing potato dextrose agar (PDA) medium. Each Petri plate contained 15 ml of PDA with 1 drop of lactic acid (0.03 ml). Five seeds were inoculated in each Petri plates and incubated for 10 days at 25±2°C in an incubator. After 5-7 days of incubation, the fungi associated with cotton seeds were recorded.

In Blotter method, a moist chamber was made by placing 2 layers of filter paper at the bottom of 9 cm diameter Petri plates, sufficient water was added to soak the blotting papers and then covered with the upper part of the Petri plate. The Petri plates were sterilized within an autoclave at 121°C for 20 minutes. Surface sterilized seeds were inoculated in Petri plates and each Petri plate contained 10 seeds. The inoculated Petri plates were incubated in an incubator (25±2°C) for 10 days. After incubation, the fungi associated with the seeds were recorded carefully.

Per cent frequency of the occurrence of the fungal isolates was calculated by adopting the following formula<sup>17</sup>.

% frequency

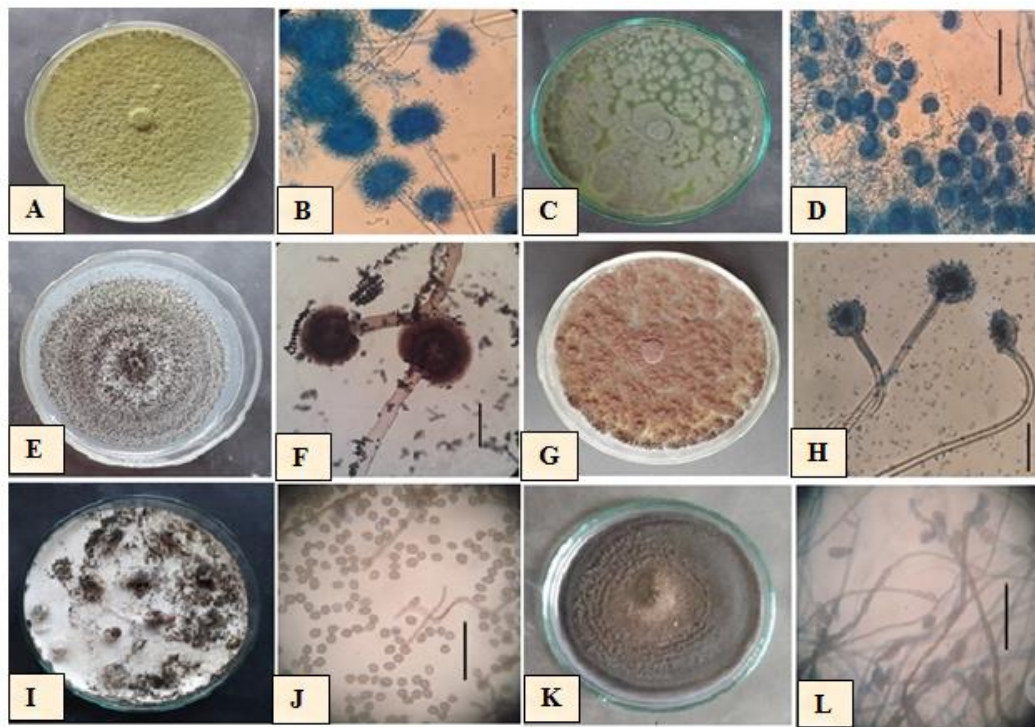
$$= \frac{\text{Total number of inocula from which a fungal isolate was observed}}{\text{Total number of inocula}} \times 100$$

### Identification of fungi

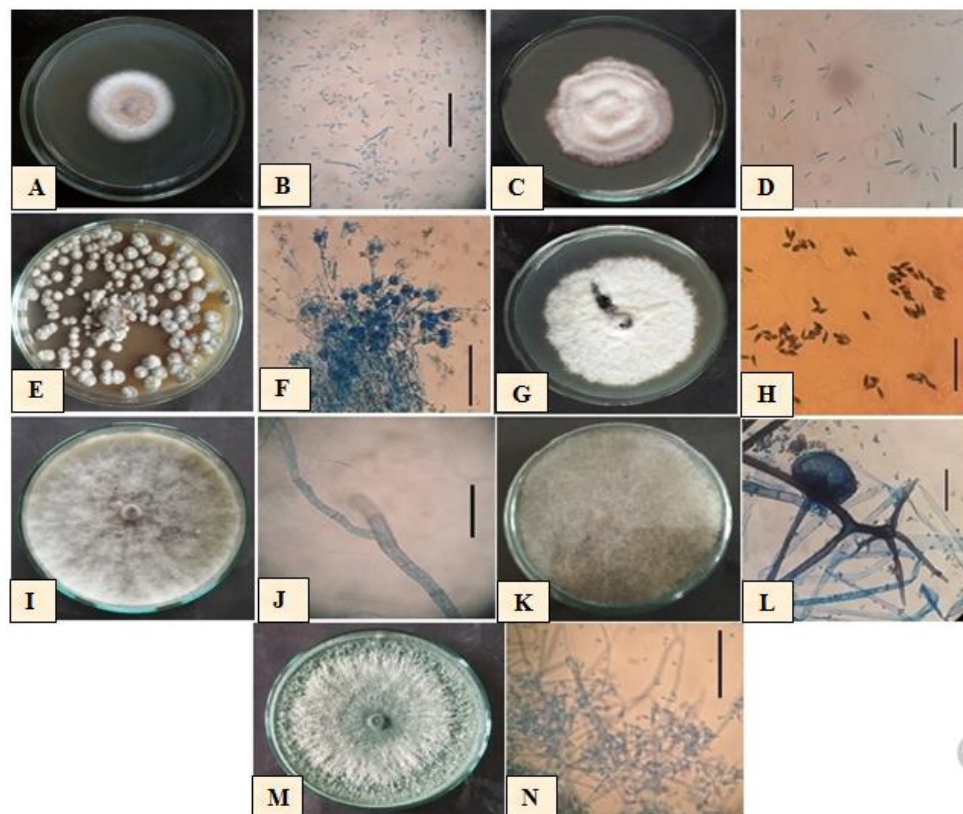
Microphotographs of the isolated fungi were taken with Nikon digital camera. Identification of the fungal isolates was determined based on morphological characteristics observed under a compound microscope following the standard literatures<sup>18-26</sup>.

## RESULTS AND DISCUSSION

A total of 12 species of fungi were isolated from three varieties of cotton seeds following Tissue planting and Blotter methods. They were *Aspergillus flavus*, *A. fumigatus*, *A. niger* (Type-I), *A. niger* (Type-II), *Chaetomium globosum*, *Curvularia lunata*, *Fusarium moniliforme* var. *subglutinans*, *Fusarium sporotrichioides*, *Penicillium* sp., *Pestalotiopsis guepinii*, *Rhizoctonia solani*, *Rhizopus stolonifer* and *Trichoderma viride* (Figs. 1 & 2).



**Fig. 1.** Culture plate, Conidiophores and conidia of *Aspergillus flavus* (A-B), *A. fumigatus* (C-D), *A. niger* (Type-I) (E-F), *A. niger* (Type-II) (G-H), Culture plate and conidia of *Chaetomium globosum* (I-J) and culture plate, Conidiophores and conidia of *Curvularia lunata* (K-L), (Bar = 50  $\mu$ m).



**Fig. 2.** Culture plate, Conidiophores and conidia of *Fusarium moniliforme* var. *subglutinans* (A-B), *Fusarium sporotrichioides* (C-D), *Penicillium* sp. (E-F), *Pestalotiopsis guepinii* (G-H); Culture plate and Sterile mycelia of *Rhizoctonia solani* (I-J), Culture plate, Sporangium with sporangiospores of *Rhizopus stolonifer* (K-L) and *Trichoderma viride* (M-N), (Bar = 50 $\mu$ m).

In Tissue planting method, a total of 9 species of fungi were isolated from HC-1 variety in two storage conditions (Table-1). They were *Aspergillus flavus*, *A. fumigatus*, *A. niger* (Type-I), *A. niger* (Type-II), *C. lunata*, *F. moniliforme* var. *subglutinans*, *F. sporotrichioides*, *P. guepinii*, *R. solani* and *R.*

*stolonifer*. At room temperature 7 fungal species were found except *P. guepinii* and *R. stolonifer* whereas all 9 fungal species were isolated at 4°C temperature. Among these fungi, *Aspergillus niger* (Type-I), *A. niger* (Type-II) and *Rhizoctonia solani* were predominant in both storage conditions (Table 1).

**Table 1.** Percent frequency of fungi associated with three varieties of cotton seeds (HC-1, HC-2 and HC-3) at different storage conditions and intervals in Tissue planting method.

| Isolated Fungi                                       | Storage Conditions | Percent frequency of isolated fungi at different intervals from three varieties of cotton seeds |                                           |                                            |       |                                           |                                           |                                            |       |                                           |                                           |                                            |       |
|------------------------------------------------------|--------------------|-------------------------------------------------------------------------------------------------|-------------------------------------------|--------------------------------------------|-------|-------------------------------------------|-------------------------------------------|--------------------------------------------|-------|-------------------------------------------|-------------------------------------------|--------------------------------------------|-------|
|                                                      |                    | HC-1                                                                                            |                                           |                                            |       | HC-2                                      |                                           |                                            |       | HC-3                                      |                                           |                                            |       |
|                                                      |                    | 1 <sup>st</sup> isolation (after 30 days)                                                       | 2 <sup>nd</sup> isolation (after 90 days) | 3 <sup>rd</sup> isolation (after 180 days) | Mean  | 1 <sup>st</sup> isolation (after 30 days) | 2 <sup>nd</sup> isolation (after 90 days) | 3 <sup>rd</sup> isolation (after 180 days) | Mean  | 1 <sup>st</sup> isolation (after 30 days) | 2 <sup>nd</sup> isolation (after 90 days) | 3 <sup>rd</sup> isolation (after 180 days) | Mean  |
| <i>Aspergillus flavus</i>                            | RT                 | -                                                                                               | 11.9                                      | -                                          | 3.97  | -                                         | 8.51                                      | -                                          | 2.84  | 8.16                                      | 32                                        | 22.45                                      | 20.87 |
|                                                      | 4°C                | 4.0                                                                                             | 8.16                                      | -                                          | 4.05  | 6                                         | 8.89                                      | -                                          | 4.96  | 10                                        | 6                                         | 8.33                                       | 8.11  |
| <i>A. fumigatus</i>                                  | RT                 | -                                                                                               | 4.76                                      | -                                          | 1.57  | 6                                         | 8.51                                      | 10                                         | 8.17  | 4.08                                      | 8                                         | -                                          | 4.03  |
|                                                      | 4°C                | -                                                                                               | 4.08                                      | -                                          | 1.36  | -                                         | -                                         | -                                          | 0     | -                                         | 2                                         | -                                          | 0.67  |
| <i>A. niger</i> (Type-I)                             | RT                 | 10.42                                                                                           | 19.05                                     | 15.0                                       | 14.82 | 12                                        | 28.4                                      | 22                                         | 20.08 | 10.20                                     | 6                                         | 10.20                                      | 8.8   |
|                                                      | 4°C                | 14.0                                                                                            | 6.12                                      | 9.09                                       | 9.74  | 14                                        | 15.56                                     | 24                                         | 23.85 | 8                                         | 8                                         | 4.17                                       | 6.72  |
| <i>A. niger</i> (Type-II)                            | RT                 | 31.25                                                                                           | 28.57                                     | 57.5                                       | 39.11 | 26                                        | 44.68                                     | 42                                         | 37.56 | 22.45                                     | 24                                        | 40.82                                      | 29.09 |
|                                                      | 4°C                | 34.0                                                                                            | 32.65                                     | 36.36                                      | 34.34 | 28                                        | 28.89                                     | 26                                         | 27.63 | 20                                        | 30                                        | 27.08                                      | 25.69 |
| <i>Curvularialunata</i>                              | RT                 | 14.58                                                                                           | 7.14                                      | -                                          | 7.24  | 12                                        | 8.51                                      | -                                          | 6.84  | 8.16                                      | 8                                         | 6.12                                       | 7.43  |
|                                                      | 4°C                | -                                                                                               | 4.08                                      | -                                          | 1.36  | 6                                         | 4.44                                      | -                                          | 3.48  | 6                                         | 6                                         | -                                          | 4     |
| <i>Fusarium moniliforme</i> var. <i>subglutinans</i> | RT                 | 4.17                                                                                            | 7.14                                      | -                                          | 3.77  | 4                                         | -                                         | 4                                          | 2.67  | 10.20                                     | 10                                        | -                                          | 6.73  |
|                                                      | 4°C                | 10.0                                                                                            | -                                         | 9.09                                       | 6.36  | 6                                         | -                                         | 6                                          | 4     | 10                                        | -                                         | 8.33                                       | 6.11  |
| <i>Fusarium sporotrichioides</i>                     | RT                 | 8.33                                                                                            | 4.76                                      | -                                          | 4.36  | 6                                         | -                                         | 2                                          | 2.67  | 6.12                                      | 2                                         | -                                          | 2.71  |
|                                                      | 4°C                | 4.0                                                                                             | 2.04                                      | -                                          | 2.01  | -                                         | -                                         | -                                          | 0     | 8                                         | 4                                         | 6.25                                       | 6.08  |
| <i>Penicillium</i> sp.                               | RT                 | -                                                                                               | -                                         | -                                          | 0     | 8                                         | -                                         | -                                          | 2.67  | 6.12                                      | -                                         | 20.41                                      | 8.84  |
|                                                      | 4°C                | -                                                                                               | -                                         | -                                          | 0     | -                                         | -                                         | -                                          | 0     | 4                                         | 4                                         | -                                          | 2.67  |
| <i>Pestalotiopsis guepinii</i>                       | RT                 | -                                                                                               | -                                         | -                                          | 0     | -                                         | -                                         | -                                          | 0     | -                                         | -                                         | -                                          | 0     |
|                                                      | 4°C                | 8.0                                                                                             | -                                         | -                                          | 2.67  | -                                         | -                                         | -                                          | 0     | -                                         | -                                         | -                                          | 0     |
| <i>Rhizoctonia solani</i>                            | RT                 | 31.25                                                                                           | 16.67                                     | 25.0                                       | 24.31 | 16                                        | 6.38                                      | 10                                         | 10.79 | 24.49                                     | 14                                        | -                                          | 12.83 |
|                                                      | 4°C                | 26.0                                                                                            | 28.57                                     | 34.09                                      | 29.55 | 18                                        | 31.11                                     | 24                                         | 24.37 | 24                                        | 40                                        | 35.42                                      | 33.14 |
| <i>Rhizopus stolonifer</i>                           | RT                 | -                                                                                               | -                                         | -                                          | 0     | 10                                        | -                                         | 10                                         | 6.67  | -                                         | -                                         | -                                          | 0     |
|                                                      | 4°C                | -                                                                                               | 14.29                                     | 11.36                                      | 8.55  | 14                                        | 11.11                                     | 20                                         | 15.04 | 10                                        | -                                         | 10.42                                      | 6.81  |
| <i>Trichoderma viride</i>                            | RT                 | -                                                                                               | -                                         | -                                          | 0     | -                                         | -                                         | -                                          | 0     | -                                         | -                                         | -                                          | 0     |
|                                                      | 4°C                | -                                                                                               | -                                         | -                                          | 0     | 8                                         | -                                         | -                                          | 2.67  | -                                         | -                                         | -                                          | 0     |

RT= Room Temperature (25°C), “-” = respective fungus did not show mycelial growth.

A total of 10 fungal species were isolated from HC-2 variety by Tissue planting method (Table 1). They were *Aspergillus flavus*, *A. fumigatus*, *A. niger* (Type-I), *A. niger* (Type-II), *C. lunata*, *F. moniliforme* var. *subglutinans*, *F. sporotrichioides*, *Penicillium* sp., *R. solani*, *R. stolonifer* and *T. viride*.

At room temperature 9 species of fungi were found to be associated with seeds of HC-2 variety except *Trichoderma viride* while at 4°C temperature 7 species of fungi were isolated except *A. fumigatus*, *F. sporotrichioides* and *Penicillium* sp. (Table 1). Among these fungi, *Aspergillus niger* (Type-I), *A. niger* (Type-II) and *Rhizoctonia solani* were predominant in both storage conditions (Table 1).

A total of 9 species of fungi were isolated from HC-3 variety following Tissue planting method (Table 1).

They were *Aspergillus flavus*, *A. fumigatus*, *A. niger* (Type-I), *A. niger* (Type-II), *C. lunata*, *F. moniliforme* var. *subglutinans*, *F. sporotrichioides*, *Penicillium* sp., *R. solani* and *R. stolonifer*. At room temperature 8 species of fungi were identified from the seeds of HC-3 variety except *Rhizopus stolonifer* whereas all 9 species of fungi were observed at 4°C temperature. Among these fungi, *Aspergillus niger* (Type-II) (29.09%), *Aspergillus flavus* (20.87%) and *Rhizoctonia solani* (12.83%) were dominant at room temperature (Table 1). *Rhizoctonia solani* (33.14%), *Aspergillus niger* (Type-II) (25.69%) and *Aspergillus flavus* (8.11%) were dominant at 4°C temperature (Table 1).

Arabsalmani<sup>27</sup> isolated *Rhizoctonia solani*, *Aspergillus flavus*, *Alternaria macrospora*, *A. alternata* and

*Fusarium* spp., from the seeds coat of cotton following Tissue planting method.

In Blotter method, a total of 5 species of fungi were isolated from HC-1, HC-2 and HC-3 varieties of cotton seeds (Table 2).

**Table 2.** Percent frequency of fungi associated with three varieties of cotton seeds in “Blotter method”.

| Cotton varieties | Storage conditions | Name of fungi    |                     |                          |                           |                            |                          |
|------------------|--------------------|------------------|---------------------|--------------------------|---------------------------|----------------------------|--------------------------|
|                  |                    | <i>A. flavus</i> | <i>A. fumigatus</i> | <i>A. niger</i> (Type-I) | <i>A. niger</i> (Type-II) | <i>Chaetomium globosum</i> | <i>Curvularia lunata</i> |
| HC-1             | RT                 | 14.3             | -                   | 9.52                     | 14.29                     | 57.14                      | 4.76                     |
|                  | 4°C                | 5                | -                   | 25                       | 20                        | 50                         | -                        |
| HC-2             | RT                 | -                | -                   | 14.71                    | 32.35                     | 47.06                      | 5.88                     |
|                  | 4°C                | -                | 16.67               | 20                       | 10                        | 53.33                      | -                        |
| HC-3             | RT                 | 10               | 15                  | 20                       | 10                        | 40                         | 5.88                     |
|                  | 4°C                | 15               | -                   | 10                       | 15                        | 70                         | -                        |

RT= Room Temperature (25°C), “-” = respective fungus did not show mycelial growth.

The isolated fungi were *Aspergillus flavus*, *A. fumigatus*, *A. niger* (Type-I), *A. niger* (Type-II), *Chaetomium globosum* and *Curvularia lunata*. At room temperature four, three and five different species of fungi were recorded in HC-1, HC-2 and HC-3 cotton varieties, respectively. At room temperature, in all varieties of cotton seeds, *Chaetomium globosum* showed the highest association percentage and the value were 57.14% in HC-1, 47.06% in HC-2 and 40% in HC-3 cotton varieties.

At 4°C temperature, *Aspergillus flavus*, *A. fumigatus*, *A. niger* (Type-I), *A. niger* (Type-II) and *C. globosum* were isolated from HC-1, HC-2 and HC-3 varieties of cotton seeds (Table 2). The highest association percentage was recorded for *Chaetomium globosum* in all the varieties of cotton seeds at 4°C and the percentage was 50% in HC-1, 53.33% in HC-2 and 70% in HC-3 (Table 2) cotton varieties. *Chaetomium globosum* exclusively found during isolation by Blotter method.

Tomar et al.<sup>28</sup> reported eleven fungal flora viz., *Aspergillus niger*, *A. flavus*, *Penicillium* sp., *Alteraria alternata*, *Chaetomium* spp, *Rhizopus niger*, *Fusarium solani*, *Macrophomina phaseolina*, *Myrothecium roridum*, *Trichothecium roseum* and *Curvularia lunata* from JK 4 cotton cultivar growing in the locations of Madhya Pradesh by Blotter method.

From the above discussion, it was observed that storage temperature and duration did not show remarkable effect on frequency of occurrence of fungi associated with cotton seeds.

A comprehensive study of literatures revealed that *Fusarium sporotrichioides* has not been reported in any relevant literatures in Bangladesh<sup>29-32</sup>. Hence, *Fusarium sporotrichioides* Sherb., Mem. is reported here as first time from Bangladesh.

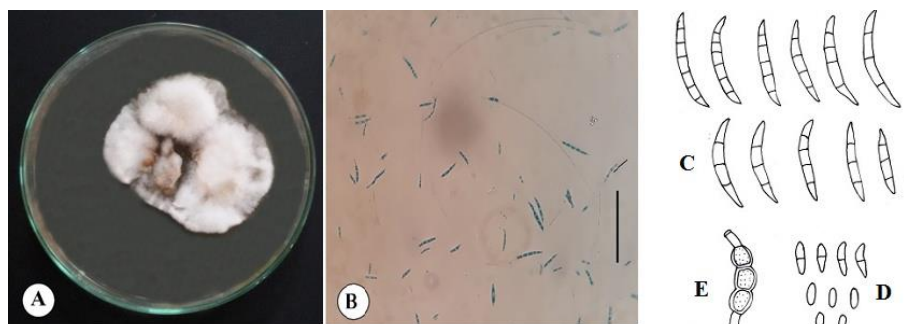
The Taxonomic descriptions of *Fusarium sporotrichioides* is given bellow:

***Fusarium sporotrichioides*** Sherb., Mem. Cornell Univ. agric. Exp. Stn. 6: 183 (1915) (**Fig. 3**)

Colonies white, aerial mycelium floccose, reverse brownish red. Mycelia hyaline, septate, profusely branched. Chlamydospore present. Phialides cylindrical, microconidia hyaline, ellipsoidal to obovate becoming one septate, 7-12 × 3-5µm. Macroconidia hyaline, falcate to curved, fusoid, 3 to 6 septate, 28-48 × 3.6-5 µm.

The fungus was isolated from seeds of all the cotton varieties studied.

Specimen examined: Isolated from HC-1 (*Gossypium arboreum* L.) MN Nahar 08, 07 May 2017.



**Fig. 3.** *Fusarium sporotrichioides*: Colony on PDA medium (A) and Conidiophores with conidia (B). (Bar = 50 µm); Camera Lucida of Macroconidia (C), Microconidia (D) and Chlamydospore (E). (Bar = 11µm).

In the present study, 12 species of fungi were isolated from the seeds of three cotton varieties (HC-1, HC-2 and HC-3). Among these fungi *Aspergillus niger* (Type-I), *A. niger* (Type-II), *Chaetomium globosum* and *Rhizoctonia solani* were predominant for seeds of all cotton.

varieties. *Fusarium sporotrichioides* is a new record for Bangladesh. Present findings will be helpful for designing the management protocol of mycoflora of cotton seeds in storage.

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