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FUNGI ASSOCIATED WITH DIFFERENT PARTS OF COTTON SEED[1]

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Abstract

Seed samples with highest (CB10), lowest (CB8), moderate fungal frequency (CB3) and randomly selected seeds from all varieties (CB1-14) were used. A total of 14 species of fungi, namely *Aspergillus flavus* Link, *A. fumigatus* Fresenius, *A. niger* Van Tiegh, *Curvularia lunata* (Wakker) Boedijn, *Colletotrichum gloeosporioides* Penz & Sacc, *Fusarium nivale* (Fr.) Sorauer, *F. oxysporum* Schlechtendal, *F. fujikuroi* Nirenberg, *Penicillium aculeatum* Raper & Fennell, *Penicillium citrinum* Thom, *Rhizoctonia solani* Khun., *Rhizopus stolonifer* (Ehrenb.) Vuill., *Syncephalastrum racemosum* Cohn and *Trichoderma viride* Pers. were found to be associated with seed parts of different varieties of cotton. The maximum ten species of fungi were isolated from seed coat. The most prevalent fungus was *Rhizopus stolonifer* which was found in all the parts of all the varieties of cotton seeds examined. Out of the 14 isolated fungi, *Aspergillus flavus, A. fumigatus*, *A. niger*, *C. lunata*, *C. gloeosporioides, F. nivale, F. oxysporum*, *F. fujikuroi, Penicillium aculeatum*, *R. solani*, *R. stolonifer*, *S. racemosum* and *T. viride* were found to be external and *Aspergillus flavus, A. fumigatus*, *A. niger*, *C. lunata*, *F. nivale, Penicillium citrinum* and *R. stolonifer* were internal seed borne fungi. *Aspergillus flavus, A. fumigatus*, *A. niger*, *C. lunata*, *F. nivale* and *R. stolonifer* were isolated as both external and internal seed borne fungi.

Introduction

Along with food and shelter, clothing is one of the primary requirements of human beings. Cotton (*Gossypium* spp.), unique among agricultural crops, provides food, animal feed, fiber, edible oil and fuel. The word cotton refers to four species of genus *Gossypium* (Malvaceae), namely *G. hirsutum* L., *G. arboreum* L., *G. herbaceum* L. and *G. barbadense* L. Two types of cotton are mainly grown in Bangladesh. They are: (i) Upland cotton or American cotton (*Gossypium hirsutum*) and (ii) Hill cotton or Comilla cotton (*Gossypium arboreum*).

Cotton is the second important cash crop next to jute in Bangladesh. It is the main source of raw materials in textile industry. It plays a key role of national economy in terms of generating direct and indirect employment in the agricultural and industrial sectors. As such it has huge prospect in Bangladesh.

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Cotton is generally propagated by seeds and these are potential harbor of numerous micro-fungi which may impair seed germination resulting in the production of abnormal seedlings[1-2]. Most of the cotton diseases are transmitted through seed which in most cases affect the quality of the fiber. Seed-borne fungi are responsible for both pre- and post emergence decay of grains, affect seedling vigor, and thus reduce the rate of seed germination and also create variation in plant morphology[3-5].

A. niger, A. flavus, A. fumigatus, A. terreus, Rhizopus stolonifer, Penicillium corylophilum, A. terreus and *A. nidulans* were isolated as the most common fungal species by Mazen *et al.*[6] from Egyptian cotton seeds. *Aspergillus flavus, A. fumigatus, A. niger, Chaetomium globosum, Penicillium* sp. and *Rhizopus stolonifer* were found to be most predominant fungi in cotton seeds (*Gossypium hirsutum* L.) in Bangladesh[7].

By weight, in cotton seeds there are 60% endosperm, 32% seed coat and 8% embryo. Fungal pathogens may be associated with cotton seeds externally or internally[8]. Seeds are the efficient medium for survival and dissemination of plant pathogens. The lack of high quality seeds and the prevalence of seed borne organisms are the main constraints in maintaining the crop production. Lot of research has been done home and abroad on cotton diseases and its control but very little information is available about the fungi associated with different parts of cotton seeds. So considering the importance of this fiber crop, present work was undertaken to investigate the prevalence of fungi in different parts of the upland cotton (*Gossypium hirsutum* L.) seeds.

Materials and Methods

The present investigation was carried on seeds of upland cotton (*Gossypium hirsutum* L.). Seed samples of CB1-14 were collected from Cotton Research, Training and Seed multiplication Farm, Sreepur, Gazipur on July, 2017 immediately after harvesting and kept in clean glass jars, labeled properly and preserved at room 25 ± 2^{0}C for subsequent investigation. The study was made at the Mycology and Plant Pathology Laboratory, Department of Botany, University of Dhaka during June - September, 2018. Here the seed samples with highest (CB10), lowest (CB8), moderate fungal frequency (CB3) and seeds selected randomly from all varieties (CB1-14) were used for the study.

The fungi were isolated from the samples following the “Tissue Planting method” on PDA medium[9]. Three hundred seeds of each sample were used in this method. Isolated fungi were transferred to separate PDA plates and PDA slants for further studies and preservation. The location of fungi in cotton seeds was studied by employing Component plating technique[10]. Cotton seed has three parts : Seed coat, embryo and endosperm (Fig. 1). Three hundred seeds of each variety were taken for study. The seed parts were separated and then surface sterilized with 10% Chlorox solution for five minutes. These parts were again washed with distilled water for three times and soaked with sterilized filter paper. The separated seed parts were then placed in Petri plates containing

sterilized potato dextrose agar (PDA) medium. Each Petri plate contained 15 ml of PDA medium with an addition of one drop (ca. 0.03 ml) of lactic acid which was used for checking the bacterial growth. Then the inoculated plates were incubated at room temperature (25 ± 2 °C) for 5 -7 days. The fungi isolated from different seed parts were examined under electron microscope.

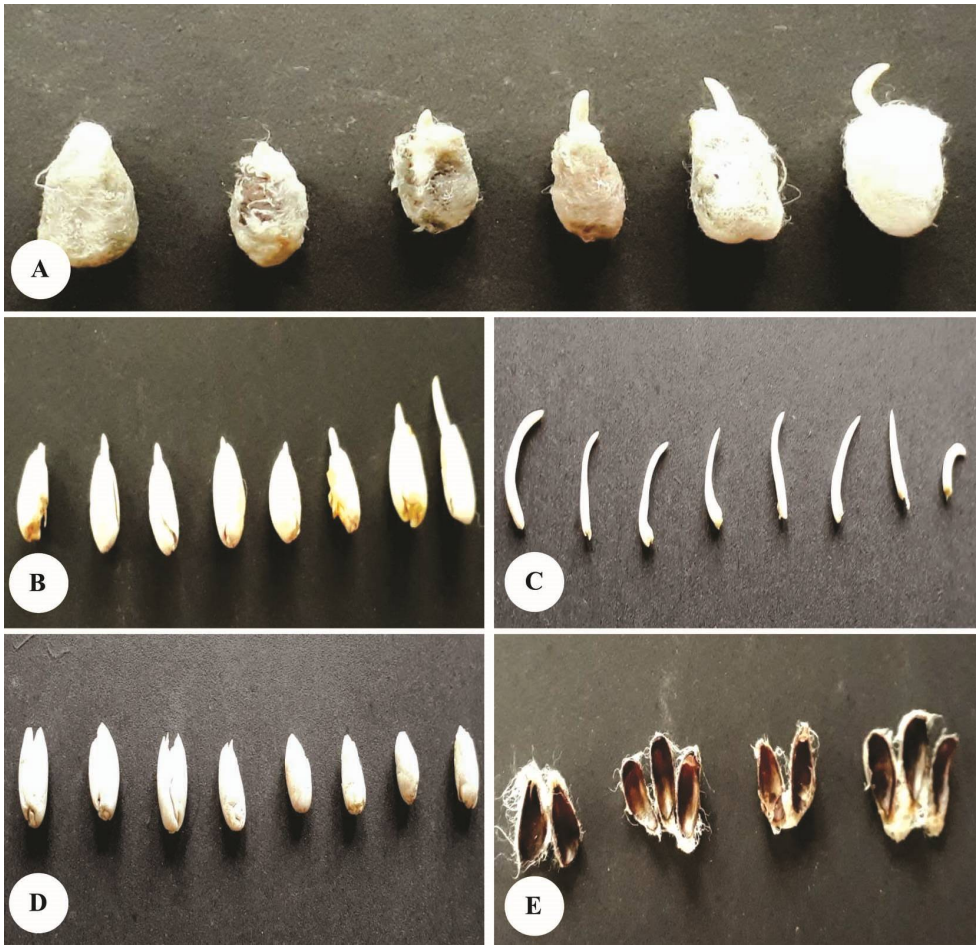

Fig. 1. Different parts of cotton seeds: A. Germinating seeds, B. Embryo with endosperm C. Embryo, D. Endosperm and E. Seed coat.

The isolated fungi were identified on the basis of morphological characteristics observed under a compound microscope following standard literature[11-19]. Molecular identification of the fungi was made following Amer *et al.*[20] with some modification. Per cent frequency of occurrence of the fungi was calculated following the formula of Spurr and Wetly[21].

Data were evaluated by ANOVA using STAR statistical program and means were compared with the help of DMRT.

Results and Discussion

All the 14 species of fungi were found to be associated with 3 parts of different varieties of upland cotton seeds (Fig. 2). Maximum numbers of fungi were found on seed coat in all cotton varieties.

Per cent frequency of fungi associated with different parts of cotton seeds (randomly selected seeds of 14 varieties) are shown in Table 1. Ten species of fungi were isolated from these categories, out of which 9 species were found in seed coat. The total frequency of *Aspegillus niger* was the highest (246) and *Penicillium citrinum* was the lowest (4). But frequency of total fungi was higher (218) in endosperm and in embryo it was lower (146). *Aspergillus flavus, A. niger* and *R. stolonifer* were found in all parts of cotton seeds whereas *C. gloeosporioides, C. lunata, F. oxysporum* and *F. fujikuroi* were found exclusively in seed coat and *Penicillium citrinum* was found only in embryo (Table 1).

Cotton variety (CB10) showed highest incidence of fungal frequency. Nine species of fungi were isolated from the aforesaid cotton varieties, of which 8 were found on seed coat. The total frequency of *R. stolonifer* was the highest (94) and *Pencillium citrinum* was the lowest (4). On the other hand, total infection in seed coat was highest (119) and in embryo it was lowest (34). In this case, *R. stolonifer* was found in all parts of cotton seeds and rests were found in only seed coat except *A. flavus* and *F. nivale* (Table 2).

Cotton variety (CB8) showed the lowest fungal frequency. Eleven fungal species were associated with the above mentioned cotton variety of which 10 were isolated from seed coat. The total frequency of *Fusarium nivale* was the highest (130) and *Penicillium aculeatum* was the lowest (3). The total fungal infection was highest in seed coat (160) and lowest in embryo (81). Here, also *R. stolonifer* was observed in all the parts of cotton seeds whereas, *F. fujikuroi*, *P. aculeatum*, *S. racemosum* and *T. viride* were found in only seed coat and *P. citrinum* in only embryo (Table 2).

Cotton variety (CB3) showed moderate fungal infection. Seven species of fungi were isolated from this variety and six were associated with seed coat. The total frequency of *R. stolonifer* was highest (167) and *F. fujikuroi* was lowest (7). Total fungal infection was higher in embryo (104) and lower in seed coat (99). *Aspergillus flavus* and *R. stolonifer* were noticed in all the parts of selected cotton seeds whereas, *A. fumigatus*, *F. fujikuroi* and *R. solani* were noticed only in seed coat of cotton seeds (Table 2).

The most prevalent fungus was *R. stolonifer* which was found in all the parts of all cotton varieties whereas, *C. gloeosporioide, F. oxysporum, Penicillium aculeatum*, *S. racemosum* and *T. viride* were found only in seed coat. The total infection was highest (218) in endosperm of randomly selected seeds of 14 different cotton varieties and lowest (34) in embryo of CB10. Out of these 14 fungi, *Aspergillus flavus, A. fumigatus*, *A. niger*,

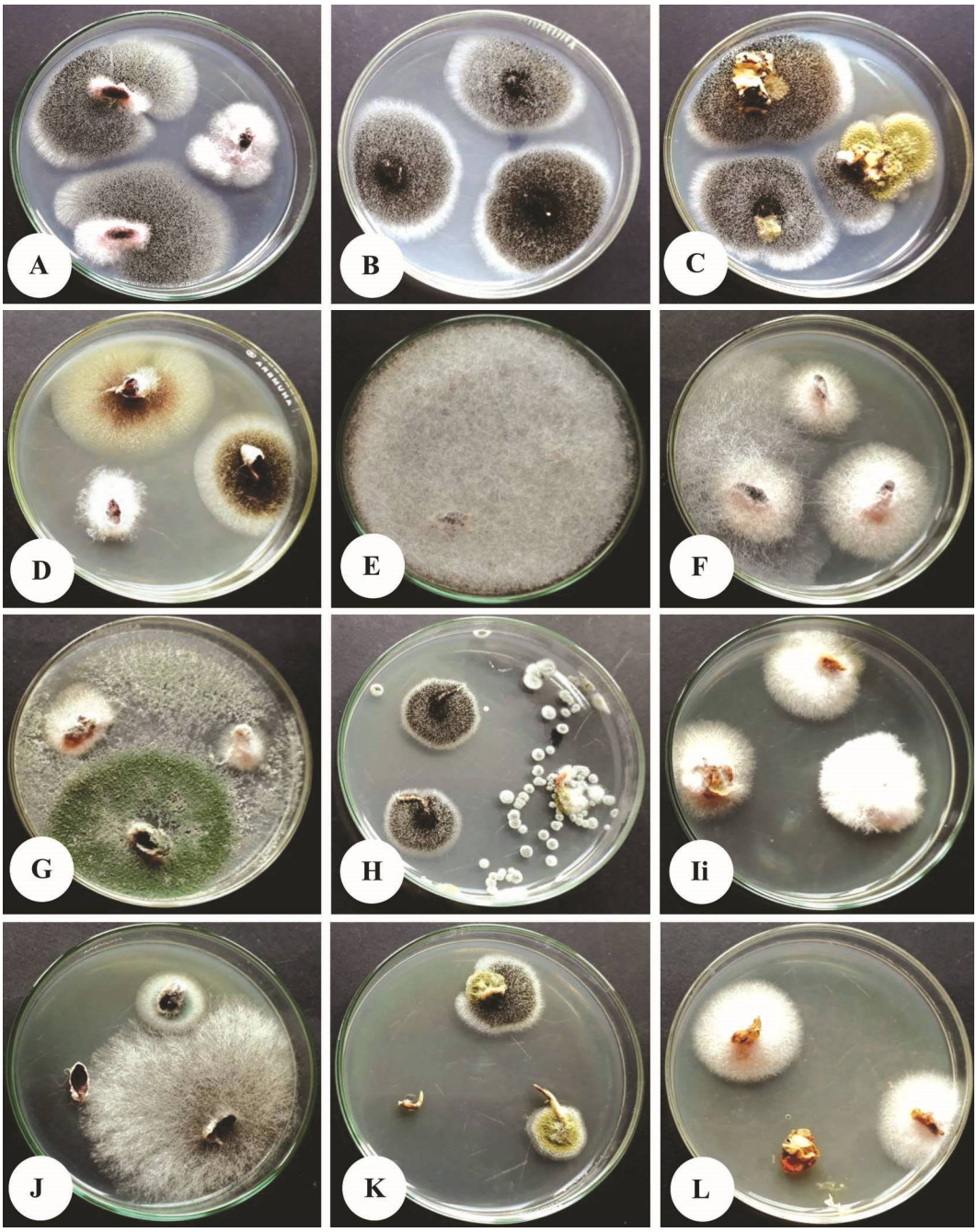

Fig. 2. Fungi associated with different parts (Seed coat, embryo and endosperm) of upland cotton seeds. A - C. Seeds randomly selected from 14 cotton varieties, D - F. CB10 with highest fungal frequency, G - I. CB8 with lowest fungal frequency and J - L. CB3 with moderate fungal frequency.

Table 1. Per cent frequency of fungi associated with different parts of cotton seeds (randomly selected seeds of 14 cotton varieties).

Name of fungi	Seed coat	Embryo	Endosperm	Mean	Total
Aspergillus fumigatus	4[g]	4[d]	0[e]	2.67	8
Aspergillus niger	74[a]	78[a]	94[a]	82.0	246
Colletotrichum gloeosporioides	7[f]	0[e]	0[e]	2.33	7
Curvularia lunata	10[e]	0[e]	0[e]	3.33	10
Fusarium fujikuroi	30[c]	0[e]	0[e]	10.0	30
Fusarium nivale	10[e]	0[e]	10[d]	6.67	20
Fusarium oxysporum	7[f]	0[e]	0[e]	2.33	7
Penicillium citrinum	0[h]	4[d]	0[e]	1.33	4
Rhizopus stolonifer	50[b]	34[b]	34[c]	39.34	118
Mean	20.9	14.6	21.8		
Total	209	146	218		
CV (%)	4.56	4.84	2.85		

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

Table 2. Per cent frequency of fungi associated with different parts of cotton seeds.

Name of fungi	CB1-14			CB10			CB8			CB3		
	SC	EM	EN	SC	EM	EN	SC	EM	EN	SC	EM	EN
Aspergillus flavus	17[d]	26[c]	80[b]	7[e]	0[c]	10[c]	10[c]	17[b]	0[d]	7[d]	27[d]	10[c]
Aspergillus fumigatus	4[g]	4[d]	0[e]	10[d]	0[c]	0[d]	3e	3[d]	0[d]	10[c]	0[b]	0[e]
Aspergillus niger	74[a]	78[a]	94[a]	40[a]	0[c]	0[d]	12[c]	7[c]	0[d]	0[e]	10[c]	7[d]
Colletotrichum gloeosporioides	7[f]	0[e]	0[e]	0[f]	0[c]	0[d]	0[f]	0[e]	0[d]	0[e]	0[d]	0[e]
Curvularia lunata	10[e]	0[e]	0[e]	7[e]	0[c]	0[d]	7[d]	0[e]	3[c]	0[e]	0[d]	0[e]
Fusarium fujikuroi	30[c]	0[e]	0[e]	4[e]	0[c]	0[d]	11[c]	0[e]	0[d]	7[d]	0[d]	0[e]
F. nivale	10[e]	0[e]	10[d]	24[b]	0[c]	17[b]	60[a]	0[e]	70[a]	11[c]	0[d]	34[b]
F. oxysporum	7[f]	0[e]	0[e]	0[f]	0[c]	0[d]	0[f]	0[e]	0[d]	0[e]	0[d]	0[e]
Penicillium citrinum	0[h]	4[d]	0[e]	0[f]	4[b]	0[d]	0[f]	4[d]	0[d]	0[e]	0[d]	0[e]
Penicillium aculeatum	0[h]	0[e]	0[e]	0[f]	0[c]	0[d]	3[e]	0[e]	0[d]	0[e]	0[d]	0[e]
Rhizoctonia solani	0[h]	0[e]	0[e]	7[e]	0[c]	0[d]	0[f]	0[e]	0[d]	14[b]	0[d]	0[e]
Rhizopus stolonifer	50[b]	34[b]	34[c]	20[c]	30[a]	44[a]	37[b]	54[a]	20[b]	50[a]	67[a]	50[a]
Syncephalastrum racemosum	0[h]	0[e]	0[e]	0[f]	0[c]	0[d]	7[d]	0[e]	0[d]	0[e]	0[d]	0[e]
Trichoderma viride	0[h]	0[e]	0[e]	0[f]	0[c]	0[d]	11[c]	0[e]	0[d]	0[e]	0[d]	0[e]
Total fungi	209	146	218	119	34	71	160	81	93	99	104	101
CV (%)	5.40	5.73	3.37	8.82	15.56	9.13	7.35	9.84	6.97	9.26	6.23	7.41

Means followed by the same letter within a column did not differ significantly at 5% level by DMRT.
SC - Seed coat, EM - Embryo, EN - Endosperm, 0 - No fungal growth.

C. lunata, *C. gloeosporioides, F. nivale, F. oxysporum*, *F. fujikuroi, Penicillium aculeatum*, *R. solani*, *R. stolonifer*, *S. racemosum* and *T. viride* were found to be external seed-borne fungi; *Aspergillus flavus, A. fumigatus*, *A. niger*, *C. lunata*, *F. nivale, Penicillium citrinum* and *R. stolonifer* were found to be internal seed-borne fungi. *Aspergillus flavus, A. fumigatus*, *A. niger*, *C. lunata*, *F. nivale* and *R. stolonifer* were isolated as both external and internal seed borne fungi.

This result was in conformity with Roy and Bourland[22] and Seneewong *et al*.[23] who also found that *A. niger* caused both external and internal infection in cotton seeds. *Fusarium lateritium, F. udum, F. vasintectum*, *F. bulbigenum, F. moniliforme, F. oxysporum, F. roseum, F. solani* and *F. truncatum* were also reported by them as externally as well as internally seed-borne fungi.

Eisa *et al*.[24] reported *Rhizoctonia solani* the highest pathogenic fungus isolated from seed coat of Giza-86, Giza-89 and Giza-89 cotton varieties. On the other hand, *Alternaria alternata, Fusarium solani, F. tricinectum, Penicillium* spp. and *A. niger* were isolated at the lowest numbers from cotton seed coat. *Alternaria alternata, Aspergillus niger, Fusarium acuminatum, Fusarium solani, Pythium ultimum, Rhizopus arrhizus* and *Rhizoctonia solani* were isolated by Mansoori and Hamdolahzadeh[25] from different parts of cotton seeds.

From this study, it was found that the fungi were associated with different seed parts of upland cotton in Bangladesh. This finding will help the farmers or cotton producers to control the seed associated fungi of cotton as well as for production of healthy, disease free seeds. This study will also provide useful insight to the researchers to uncover the critical areas of seed associated fungi or seed-borne fungi of upland cotton in Bangladesh.

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References

1. Bateman GL and H Kwasna 1999. Effects of number of winter wheat crops grown successively on fungal communities on wheat roots. Applied Soil Ecology **13**: 271-282.
2. Khanzada KA, MA Rajput, GS Shah, AM Lodhi and F Mehboob 2002. Effect of seed dressing fungicides for the control of seed borne mycoflora of wheat. Asian J. Plant Sci. **1**(4): 441-444.
3. Van Du P, LC Loan, ND Cuong, HV Nghiep and ND Thach 2001. Survey on seed borne fungi and its effects on grain quality of common rice cultivars in the Mekong Delta. Omonrice **9**: 107-113.
4. Rajput MA, MA Pathan, AM Lodhi, GS Shah and KA Khanzada 2005. Studies on seed-borne fungi of wheat in Sindh Province and their effect on seed germination. Pak. J. Bot. **37**(1): 181-185.
5. Niaz I and S Dawar 2009. Detection of seed-borne mycoflora in maize (*Zea mays* L.). Pak. J. Bot. **41**(1): 443-451.
6. Mazen MB, IA el-Kady and SM Saber 1990. Survey of the mycoflora and mycotoxins of cotton seeds and cotton seed products in Egypt. Mycopathologia **110** (3): 133-138.
7. Khatun A, S Shamsi and MA Bashar 2018. Diversity of seed borne fungi associated with fourteen varieties of storage cotton (*Gossypium hirsutum* L.) seeds. Journal of Biodiversity, Conservation and Bioresource Management **4**(2): 43-52.

8. Singh D and SB Mathur 2004. Location of fungal hyphae in seeds. Histopathology of seed borne infections. Boca Raton, FL, USA: CRC Press. pp. 101-168.
9. CAB (Commonwealth Agricultural Bureau) 1968. *Plant Pathologist Pocket Book*. 1st edn. The Commonwealth Mycological Institute, England. pp. 267.
10. ISTA 1996. International Rules of Seed Testing Association. *In:* Proc. Int. Seed Test. Assoc. pp. 19-41.
11. Thom C and KB Raper 1945. A Manual of the Aspergilli. Williams and Wilkins, Baltimore, M.D. USA. pp. 373.
12. Raper KB and C Thom 1949. Manual of the Penicillia, Williams and Wilkins, Baltimore, MD. USA. pp. 875.
13. Subramanian CV 1971. Hyphomycetes. Indian Council of Agriculture Research, New Delhi, pp. 930.
14. Barnett HL and SB Hunter 1972. Illustrated Genera of Imperfect Fungi. Burgess Publishing Company, USA Third Edition, pp. 44-45.
15. Benoit MA and SB Mathur 1970. Identification of species *Curvularia* on Rice Seed. Proc. Inst. Seed Test. Ass. **35**(1): 1-23.
16. Booth C 1971. The Genus *Fusarium.* The Commonwealth Mycological Institute, Kew, England, pp. 267.
17. Ellis MB 1971. Dematiaceous Hyphomycetes. The Commonwealth Mycological Institute, England, pp. 608.
18. Ellis MB 1976. More Dematiaceous Hyphomycetes. The Commonwealth Mycological Institute, England, pp. 507.
19. Sutton BC 1980. The *Coelomycetes*, Common Wealth Mycological Institute, Kew Surrey, England, pp. 696.
20. Amer *et al*. 2011. Non liquid nitrogen-based-method for isolation of DNA from filamentous fungi. African Journal of Biotechnology **10**(65): 14337-14341.
21. Spurr HWJ and RE Wetly 1972. Incidence of tobacco leaf microflora in relation to brown spot disease and fungicidal treatment. Phytopathol. **62**: 916- 920.
22. Roy KW and FM Bourland 1982. Epidemiological and mycofloral relationship in cotton seedling diseases in Mississippi. Phytopathol. **2**: 101-102.
23. Seneewong A, C Bashin and WE Baston 1991. The relationship between internal disease organisms and germination of gin run cotton seed (*Gossypium hirsutum*). J. Seed Technol. **15**(2): 91-96.
24. Eisa A, GM El-Habbaa, MF Aboul-Ella and SR Hassan 2007. Associated fungi with seeds of some Egyptian cotton cultivars and their effect on the plant mortality production and oil content, Agric. Botany Dept., Plant Pathology Branch, Fac. Agric., Benha University, Giza, Egypt. pp. 1-15.
25. Mansoori, B and A Hamdolahzadeh 1995. Seed test and seedling disease of cotton in Gorgon and Gonbad. Applied Entomology and Phytopathology **62**: 1- 217.

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