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.(6,8). 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 \textit{G. arboreum} seeds.

**Materials and Methods**

Seeds of three varieties of cotton (\textit{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 \(10^4\) /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).

![Pathogenicity test of six isolated fungi](image_url)
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 (%) | Morality (%) |
|----------------------------------|---------------------------|--------------------------|-----------------|--------------|
| Uninoculated seeds               | 85.00^a                   | 48.40^a                  | 100^a           | 3.00^e        |
| Inoculated seeds                 |                           |                          |                 |              |
| Aspergillus flavus               | 34.50^e                   | 20.83^d                  | 60.00^d         | 50.00^a       |
| A. niger (Type-I)                | 53.63^b                   | 30.00^c                  | 70.00^b         | 28.57^d       |
| Curvularia lunata                | 48.00^c                   | 37.00^b                  | 60.00^d         | 31.00^cd      |
| Fusarium moniliforme var. subglutinans | 46.00^c             | 29.57^c                  | 60.00^d         | 33.30^c       |
| F. sporotrichioides              | 40.00^d                   | 20.43^d                  | 55.00^d         | 38.00^b       |
| Rhizoctonia solani               | 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.

Kumar et al.\textsuperscript{(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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References

1. Wendel JF, C Brubaker, J Alvarez, R Cronn and JM Stewart 2009. Evolution and natural history of the cotton genus. In: Andrew H. Paterson (Ed.). Genetics and genomics of cotton. Plant genetics and genomics: Crops and Models 3: 3-22.
2. Pandey BP 1980. Economic Botany: Fibre and Fibre Yielding Plants. pp. 138-147.
3. Jiskani MM 1992. Diseases of cotton and their control. Monthly "Sindh Agriculture", Agricultural Extension Sindh, Hyderabad 2(8): 9-13.
4. Terrence LK and CS Rockroth 2001. Compendium of Cotton Diseases, 2nd edn. American Phytopathological Society. pp. 77.
5. Seneewong A, CC Bashin and WE Baston 1999. The relationship between internal disease organisms and germination of gin run cotton seed (Gossypium hirsutum). J. Seed Technology 15: 91.
6. Palmateer AJ, KS McLean, G Morgan-Jones and E Van Santen 2004. Frequency and diversity of fungi colonizing tissues of upland cotton. Mycopathologia. 157(3): 303-316.
7. Kamal M and SM Moghal 1968. Studies on plant diseases of South West Pakistan. Agricultural Research Institute, Tandojam. pp.xii + 207.
8. Hafiz A 1986. Plant diseases. PARC, Pakistan, Islamabad. pp. 552.
9. Ranney CD 1962. Fungi involved in the seedling disease complex of cotton in the Yazoo–Mississippi delta. Plant Dis. Rep. 46: 122-123.
10. Johnson LF and AY Chambers 1973. Isolation and identity of three species of Pythium that cause cotton seedling blight. Plant Dis. Rep. 57: 848-852.
11. Minton EB and RH Garber 1983. Controlling the Seedling Complex of Cotton. Plant Disease. 67(1): 115.
12. Shamsi  S, A Yasmin and RJF Lutfunnessa 2008. Association of Sclerotium rolfsii Sacc. with boll rot disease of cotton (Gossypium Hirsutum L.) var. CB3 in Bangladesh. Dhaka. Univ. J. Biol. Sci 17(2): 155-158.
13. Lutfunnessa, RJF and S Shamsi 2011. Fungal diseases of cotton plant (Gossypium hirsutum L.) in Bangladesh. Dhaka Univ. J. Biol. Sci. 20(2):139-146.
14. Shamsi S. and N Naher 2014. Boll rot of cotton (Gossypium hirsutum L.) caused by Rhizopus oryzae Went & Prins. Geerl. - A new record in Bangladesh. J. Agril. Res. 39(3):547-551.
15. Shamsi S, N Naher and R Azad. 2015. Mycoflora of cotton plant (Gossypium hirsutum L.) - with three new records of Deuteromycets from Bangladesh. J. Bangladesh Acad. Sci. 39(2): 213- 221.
16. Shamsi S, N Nahar, P Chaowdhury and S Momtaz 2010. Fungal diseases of three aromatic rice
(\textit{Oryza sativa} L.). Journal of Bangladesh Academy of Sciences 34(2): 63-70.
17. Thom C and KB Raper 1945. A manual of the Aspergilli. Williams and Wilkins, Baltimore,
M.D. USA. pp. 373.
18. Raper KB and C Thom 1949. Manual of the Penicillia, Williams and Wilkins, Baltimore, M.D.
USA. pp. 875.
19. Benoît MA and SB Mathur 1970. Identification of species \textit{Curvularia} on rice seed. Proc. Inst.
Seed Test. Ass. 35(1): 1-23.
20. Booth C 1971. The Genus \textit{Fusarium}. Commonwealth Mycological Institute, Kew, Surrey,
England. pp. 237.
21. Subramanian CV 1971. Hyphomycetes. Indian Council of Agriculture Research, New Delhi,
pp. 930.
22. Barnett HL and BB Hanter 1972. Illustrated genera of imperfect fungi. Burgess Pub. Co. USA.
pp. 241.
23. Ellis MB 1976. More Dematiaceous Hyphomycetes. The Commonwealth Mycological Institute,
England. pp. 507.
24. Chowdury P, S Shamsi and MA Bashar 2015. Grain spotting of rice caused by \textit{Pestalotiopsis
guepinii} (Desm.) Stay - A new record. Dhaka Univ. J. Biol. Sci. 24(1): 103-106.
25. Kumar K, J Singh and HK Saksena 1984. Fungi associated with sesame seed, their nature and
control. Journal of India Phytopathology 37: 330-332.

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