

TRANSMISSION OF PATHOGENIC FUNGI FROM SEEDS TO SEEDLINGS IN COTTON (*GOSSYPIUM HIRSUTUM* L.)

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Abstract

Cotton seeds of susceptible variety CB10 were inoculated with nine pathogenic fungi viz., *Aspergillus flavus*, *A. niger*, *A. tamarii*, *Colletotrichum gloeosporioides*, *Curvularia lunata*, *Fusarium nivale*, *F. moniliforme*, *Mucor* sp. and *Rhizoctonia solani*. Seedlings were grown in water agar test tube and in earthen pots. In water agar test, *R. solani* showed the highest percentage of seed to seedling transmission (35.27%) which was followed by *F. moniliforme* (31.29%) and *A. niger* showed the lowest (8.03%) transmission. Germination percentage of control seeds was 90.32% whereas in pathogen inoculated seeds it varied from 58.33 to 89.07%. The seedling mortality percentage was highest in *R. solani* (46.68%) and lowest in *C. gloeosporioides* (18.67%). Germination percentage of control seeds in pot was 99% whereas in pathogen inoculated seeds it varied from 43.37 to 84.34%. The seedling mortality percentage was highest in *F. moniliforme* (48.28%) and lowest in *A. niger* (19.64%). Percentage of seed to seedling transmission of fungi varied from 17.85% (*A. niger*) to 46.56% (*F. moniliforme*).

Introduction

Seed is a small embryonic plant which is a basic unit of production for the world's food crop. It is an efficient means of introducing plant pathogens into a new area as well as providing a means of their survival from one cropping season to another. Seed is the most important input for crop production. Pathogen free healthy seeds are essential for desired plant populations and a good harvest. Of the 16% annual crop losses due to plant diseases, at least 10% loss occurs due to seed-borne diseases⁽¹⁾.

Seed-borne fungi are the most important biotic constraints in seed production worldwide. Coincidentally, important or devastating crop diseases are seed-borne and caused by fungi. In addition, it has demonstrated that seed borne fungi are responsible for poor quality seeds in many crops⁽²⁾. Seed diseases cause seed rot and damping-off of the seedlings. Seed-borne fungi affect the quality of seeds at all stages of production, from the cropping stages until post-harvest, processing, storage and marketing⁽³⁾. Most cotton diseases are transmitted through seeds and in most cases, they affect the quality of

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the fibre and seed. Each year, cotton production is being reduced due to the presence of some infectious pathogens and most of the seed-transmitted pathogens are fungi. Majority of diseases in cotton such as *Alternaria* blight, bacterial blight, *Fusarium* wilt, *Myrothecium* blight, *Cercospora* blight, *Exserohilum* blight etc. are seed-borne. *Alternaria alternata*, *Aspergillus niger*, *Fusarium oxysporum*, *F. moniliforme*, *F. semitectum*, *F. acuminatum*, *F. solani*, *Pythium ultimum*, *Rhizopus arrhizus*, *Rhizoctonia solani* and several other fungi were also isolated from cotton seeds and most of these fungi were pathogenic to cotton⁽⁴⁻⁵⁾. In Bangladesh, *Alternaria tenuis*, *Aspergillus flavus*, *A. niger*, *A. fumigatus*, *Fusarium moniliforme* and *Rhizopus nigricans* were reported to be predominant in cotton seeds⁽⁶⁾. *Aspergillus flavus*, *A. niger*, *Curvularia lunata*, *Fusarium moniliforme* var. *subglutinans*, *F. sporotrichioides* and *Rhizoctonia solani* were found to be pathogenic for *Gossypium arboreum* in Bangladesh⁽⁷⁾. Among these seedborne pathogens occupy an important place as regard to seed quality and yield losses⁽⁸⁾.

Information on transmission of pathogens is a prerequisite for proper prevention of disease through seed treatment, setting tolerance levels for variety release, in inspection of farmers' seed production schemes, quarantine, germplasm management and exchange and optimization of storage conditions⁽⁹⁾. Successful agricultural cropping depends on quality of seeds used for sowing. Study on seed to seedling transmission of seed borne pathogenic mycoflora of upland cotton is inadequate in our country. So, considering the importance of disease infestation of this valuable fiber crop, present research work was undertaken to evaluate the seed to seedling transmission of the isolated pathogenic fungi of cotton.

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 on transmission of pathogenic fungi from seed to seedling in test tubes was done followed by Khare *et al.*⁽¹⁰⁾. Nine pathogenic seed borne fungi of cotton viz., *Aspergillus flavus* Link, *A. niger* Van Tiegh, *A. tamarii* Kita G., *Colletotrichum gloeosporioides* Penz & Sacc, *Curvularia lunata* (Wakker) Boedijn, *Fusarium nivale* (Fr.) Sorauer, *F. moniliforme* J. Sheldon, *Mucor* sp. P. Micheli ex L. and *Rhizoctonia solani* Khun. were selected for this test. Three hundred seeds of the variety CB10 was selected and soaked in distilled water in the beaker for 30 minutes and then surface sterilized with 10% Chlorox for 5 minutes. Spore suspensions of all the test fungi at 10⁴/ml concentration were prepared in separate sterilized beakers except *R. solani*. For *R. solani*, ten mycelial blocks were used for preparation of suspension to inoculate seeds. Seeds were taken in the beakers with spore suspension and then left undisturbed condition for 2 hours. The seeds were placed on the sterilized filter paper to remove the excess surface water. After

that single seed was placed in sterilized 8-inch cotton plugged test tubes containing 10 ml 2% water agar medium. Healthy seeds served as control. Thirty test tubes were used for each pathogen. The test tubes with seeds were then incubated in the laboratory at room temperature (25 ± 2 °C). Observation was made for 3 weeks at 3 days intervals. For the presence of visible symptoms (seed rot, germination failure and infection or death of emerged seedlings) developed by the pathogens present in the seeds, the germinating seeds and seedlings were examined. Germination percentage of seeds, development of disease symptoms, seedling mortality, shoot length and root length of seedlings were recorded from healthy and inoculated seeds of the cotton variety. The symptoms produced on the germinating seeds and seedlings by the associated pathogens were confirmed by examining the seeds under stereo-binocular microscope. Seedling symptoms of pathogenic fungi were recorded according to Hansraj *et al.*⁽¹¹⁾.

Seeds of selected cotton variety CB10 were grown in pots filled with sterilized soil for testing transmission of pathogenic fungi from seed to seedlings in pot culture⁽¹¹⁾. Previously mentioned nine pathogenic fungi were also used for this experiment. Six hundred seeds of the variety were selected and soaked in distilled water for 30 minutes and then surface sterilized with 10% Chlorox solution for 5 minutes. Spore suspensions of all the test fungi were prepared in separate sterilized beakers following previously mentioned process. Seeds were placed in beakers with spore suspension and then left undisturbed condition for 2 hours. Then twenty seeds were sown per pot. Three pots and sixty seeds were used for each pathogen. In case of control, surface sterilized healthy seeds were used. The pots were kept in net house and watered regularly. Observation was made for 4 weeks at 5 days intervals and symptoms were recorded after 21 days of sowing or inoculation. Seedling symptoms owing to pathogenic fungi were recorded in water agar test and pot experiment according to Dhakar and Ratnoo⁽¹²⁾. Inoculated pathogens were re-isolated from the symptomatic area of seedlings.

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

Results revealed that all the nine test pathogens were positive and potentially infected the cotton seedlings by inducing various symptoms like root browning, root rot, stem rot, seedling blight, wilting, leaf spots etc. *Fusarium* and *Curvularia* spp. showed direct influence on the reduction of seed germination, seedling height, vigor and root length.

All the test pathogens showed seedling rot symptoms. Stunting of seedlings was also a common symptom showed for maximum pathogens. *Curvularia lunata* and *Mucor* sp. showed wilting symptom. *Aspergillus flavus*, *Curvularia lunata* and *Mucor* sp. showed blight symptoms. Healthy control seeds did not show any symptom on seedlings (Table 1, Fig. 1).

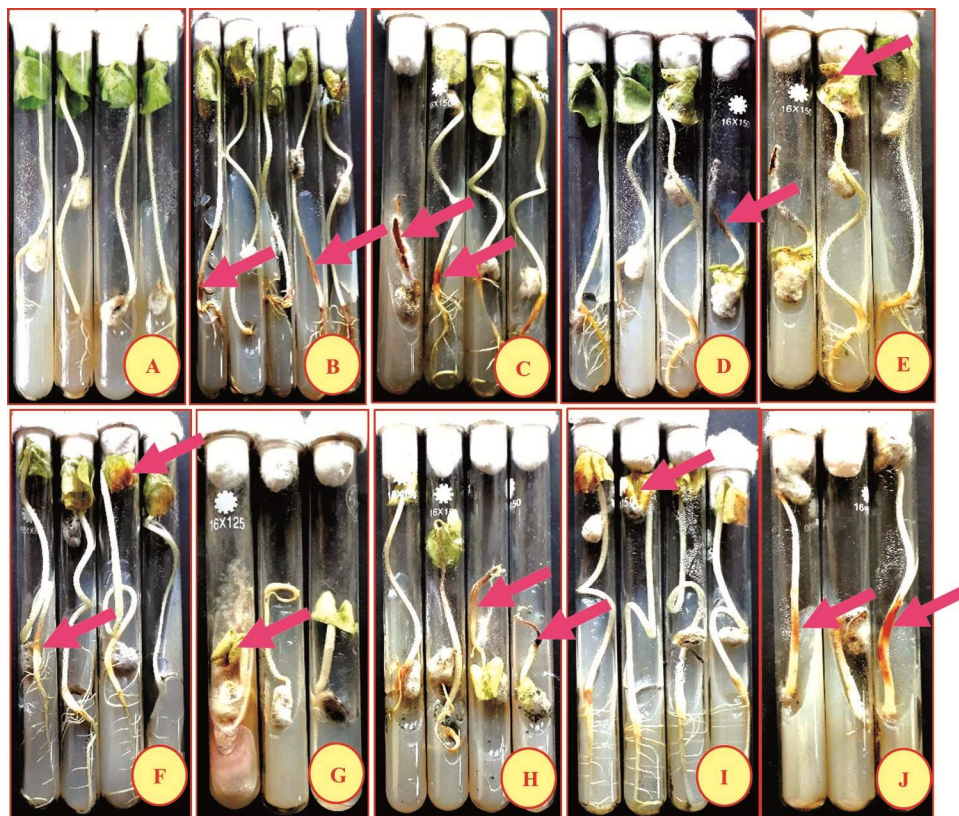


Fig. 1. Transmission of pathogenic fungi from seed to seedlings in water agar test tubes. A. Healthy seedlings and B-J. Infected seedlings caused by B. *Aspergillus flavus*, C. *A. niger*, D. *A. tamaraii*, E. *Colletotrichum gloeosporioides*, F. *Curvularia lunata*, G. *Fusarium nivale*, H. *F. moniliforme*, I. *Mucor* sp. and J. *Rhizoctonia solani*.

Rhizoctonia solani showed the higher percentage of seed to seedling transmission i.e., 35.27% which was followed by *F. moniliforme* (31.29%), *C. gloeosporioides* (22.68%), *C. lunata* (21.25%), *Aspergillus flavus* (20.38%), *A. tamaraii* (18.98%), *F. nivale* (14.89%), *Mucor* sp. (11.76%) and *A. niger* (8.03%). Germination percentage of control seeds was 90.32% whereas, in inoculated seeds it varied from 58.33 to 89.07%. The mortality percentage was highest in *Rhizoctonia solani* (46.68%) and lowest in *C. gloeosporioides* (18.67%). Seed to seedling transmission of pathogens varied from 8.03 to 35.27%. The root and shoot length of control seedlings were 6.2% and 9.78%, respectively but these values were lower for pathogens treated seeds than control (Table 1).

Yellowing of leaf followed by blight symptoms were observed in seedlings after 21 days of inoculation in pot culture experiment. *Aspergillus flavus*, *A. tamaraii*, *Colletotrichum gloeosporioides*, *Fusarium moniliforme* and *Rhizoctonia solani* showed wilting symptom. *Fusarium nivale* showed stunted growth symptom. *A. flavus*, *C. gloeosporioides*, *Curvularia*

Table 1. Transmission of test pathogens from seed to seedlings in water agar test.

Sl. No.	Test pathogens	% Germination of seeds	% mortality	% Transmission of pathogens	Length (cm)		Symptoms
					Root	Shoot	
01.	<i>Aspergillus flavus</i>	83.32 ^d	20.53 ^h	20.38 ^e	4.18 ^{fg}	5.83 ^f	Yellowing of leaves, blight, stunting, seedling rot
02.	<i>A. niger</i>	89.07 ^b	22.05 ^g	8.03 ⁱ	4.4 ^{ef}	6.51 ^e	Yellowing of leaves, stunting, root rot, stem rot
03.	<i>A. tamarii</i>	58.33 ^j	38.57 ^d	18.98 ^f	5.42 ^c	6.4 ^e	Stunting, wilting, seedling rot, yellowing of leaves
04.	<i>Colletotrichum gloeosporioides</i>	64.10 ⁱ	18.67 ^j	22.68 ^c	4.7 ^d	8.10 ^c	Stem rot, yellowing of leaves, wilting
05.	<i>Curvularia lunata</i>	76.78 ^e	41.23 ^c	21.25 ^d	5.8 ^b	8.91 ^b	Wilting, blight, seedling rot
06.	<i>Fusarium moniliforme</i>	65.57 ^h	45.32 ^b	31.29 ^b	4.58 ^{de}	6.79 ^e	Yellowing of leaves, seedling rot, root rot
07.	<i>F. nivale</i>	71.67 ^f	29.94 ^e	14.89 ^g	5.1 ^c	7.56 ^d	Stunting, yellowing of leaves, stem rot
08.	<i>Mucor</i> sp.	88.35 ^c	25.58 ^f	11.76 ^h	5.7 ^b	8.48 ^{bc}	Wilting, seedling blight, stunting, root rot.
09.	<i>Rhizoctonia solani</i>	67.32 ^g	46.68 ^a	35.27 ^a	4.14 ^g	7.54 ^d	Yellowing of leaves, seedling rot, stunting
10.	Control	90.32 ^a	16.67 ^j	-	6.2 ^a	9.78 ^a	Healthy seedlings without any symptoms
	CV (%)	0.0132	0.0328	0.0495	1.54	1.95	

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

Table 2. Transmission of test pathogens from seed to seedlings in pot experiment.

Sl. No.	test pathogens	% germination of seeds	% mortality	% Transmission of pathogens	Length (cm)		Symptoms
					Root	Shoot	
01.	<i>Aspergillus flavus</i>	79.01 ^c	27.59 ^g	25.58 ⁱ	3.39 ^b	10.93 ^b	Yellowing of leaves, seedling rot, blight, wilting
02.	<i>A. niger</i>	84.34 ^b	19.64 ⁱ	17.85 ^g	3.89 ^{cd}	11.03 ^c	Yellowing of leaves, blight
03.	<i>A. tamarii</i>	71.35 ^e	39.62 ^c	37.74 ^b	2.78 ^g	9.26 ⁱ	Wilting, blight, yellowing of leaves
04.	<i>Colletotrichum gloeosporioides</i>	58.34 ^f	35.56 ^e	33.32 ^e	3.26 ^{de}	10.73 ^e	Stem rot, leaf rot, yellowing of leaves, wilting, blight
05.	<i>Curvularia lunata</i>	43.37 ⁱ	37.04 ^d	34.33 ^d	4.0 ^a	10.09 ^f	Blight, leaf rot, stem rot
06.	<i>Fusarium moniliforme</i>	76.37 ^d	48.28 ^a	46.56 ^a	4.0 ^a	9.96 ^g	Yellowing of leaves, seedling death, blight, wilting
07.	<i>F. nivale</i>	50.56 ^h	32.02 ^f	31.20 ^f	3.14 ^{ef}	8.89 ^j	Stunting, yellowing of leaves, blight
08.	<i>Mucor</i> sp.	72.09 ^e	25.93 ^h	24.08 ^h	3.0 ^f	9.71 ^h	Blight, leaf rot, yellowing of leaves
09.	<i>Rhizoctonia solani</i>	55.67 ^g	41.86 ^b	36.88 ^c	3.36 ^{cd}	10.78 ^d	Yellowing of leaves, blight, wilting, seedling death
10.	Control	99.0 ^a	0 ^j	0 ^j	4.14 ^a	11.98 ^a	Healthy seedlings without any symptoms
	CV (%)	0.4591	0.0308	0.1143	1.61	0.0968	

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

lunata, *F. moniliforme* and *Mucor* sp. showed rot symptoms. Germination percentage of control seeds was 99% whereas, in inoculated seeds it varied from 43.37 to 84.34%. Among all the fungal pathogens, the lowest seed germination (43.37%) was recorded in pots inoculated by *Curvularia lunata*. The mortality percentage was the highest in *F. moniliforme* (48.28%) and the lowest (19.64%) in *A. niger*. Seed to seedling transmission of pathogens varied from 17.85 to 46.56% (Table 2).

Fakir *et al.*⁽¹³⁾ studied on seed transmission of *Macrophomina phaseolina* in sunflower and revealed that it caused death of the emerging radical, discoloration of roots, hypocotyls and cotyledons. Similarly, *Verticillium dahlia* transferred from seed to seedlings in cotton found by Gore *et al.*⁽¹⁴⁾. Cotton seedling diseases are a worldwide problem caused by a complex of microorganisms. Fungi are the widest pathogens which affect cotton crop especially at the seedling stage causing pre or post emergence damping off⁽¹⁵⁾.

According to Sultana⁽¹⁶⁾, six pathogenic fungi of rice *viz.*, *Bipolaris oryzae*, *Curvularia lunata*, *Fusarium equiseti*, *F. fujikuroi*, *Microdochium fisheri* and *Nigrospora oryzae* showed seed to seedling transmission nature in both water agar test tube and earthen pot. Similarly, Elwakil and Ghoneem⁽¹⁷⁾ found the transmission of four pathogenic fungi named *Fusarium oxysporum*, *F. solani*, *F. moniliforme* and *Verticillium* sp. from seed to mature plants of black cumin in Egypt. Chowdhury⁽¹⁸⁾ also observed that nine pathogenic fungi of rice were transmitted from seed to seedlings and produced different types of symptoms. The present findings are also in agreement with the results of above-mentioned researchers.

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