# PREVALENCE OF FUNGI ASSOCIATED WITH SOYBEAN SEEDS AND PATHOGENICITY TESTS OF THE MAJOR SEED-BORNE PATHOGENS

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**ABSTRACT**

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An investigation to detect the seed borne fungi of soybean and the pathogenicity tests of two major seed-borne fungal pathogens against soybean was conducted in the Microbiology Laboratory of Plant Pathology Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, during 2005 to 2006. A total of 33 soybean seed samples were collected from four different locations namely Gazipur (BARI), Mymensingh (BAU), Meherpur (local farmer) and Noakhali (local farmer) representing three varieties and 16 genotypes. Blotter method was used for detection of the associated fungi of soybean seeds. Altogether, ten fungi comprising nine genera namely *Alternaria alternata*, *Aspergillus flavus*, *Aspergillus niger*, *Chaetomium globosum*, *Colletotrichum dematium*, *Curvularia lunata*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Penicillium* sp. and *Rhizopus stolonifer* were found to be associated with the tested soybean seed samples. The germination of seed samples varied from 16-98 %. Germination of seeds was directly related to the prevalence of fungi associated with the seed. The pathogenicity test was performed with 25 isolates of *C. dematium* and 33 isolates of *F. oxysporum* against the variety Shohag in pot culture. All the tested isolates of both the pathogens were found to be pathogenic. Among the tested 25 isolates of *C. dematium* two isolates namely C19 and C20 collected from Noakhali district from variety Shohag were appeared to be virulent. Twelve were appeared to be moderately virulent while rest of the 11 isolates was found to be weak pathogen. Among the tested isolates of *F. oxysporum*, three isolates namely F21, F22 and F23 were appeared to be highly virulent. Seven isolates were found to be virulent, 13 isolates showed moderately virulence reaction and 10 isolates were appeared to be weak pathogens.

**Key words:** Pathogenicity, prevalence, seed-borne fungi, seed germination

## INTRODUCTION

Soybean, (*Glycine max* (L) Merril) is a world wide economic crop and the most important cultivated legume with hundreds of food, feed and industrial uses. It is an introduced crop in Bangladesh. The crop can be grown in tropical, sub- tropical as well as the temperate regions. It is a primary source of vegetable oil and protein concentrates (Anon. 1994). Soybean is an excellent source of major nutrients, about 40% of dry matter is protein and 20% fat (Cald Well 1973). As soybean acreage has expanded throughout the world, diseases have increased in number and severity. All parts of the soybean plant are susceptible to a number of pathogens which reduce the quality and/or quantity of seed yield. Soybean suffers from as many as 150 different diseases (Sinclair and Shurtleff 1975). Generally one or more diseases can be found in one field wherever soybeans are grown. A specific one may be very destructive in one season and difficult or impossible to find in the next season. Among the serious diseases of soybean, most of them are seed transmitted. In Bangladesh, only a limited study has been done on the prevalence of seed-borne fungi of soybean. In a preliminary report by Bhuiyan and Fakir (1982) on the important seed-borne fungi of soybean encountered were *Cercospora kikuchii*, *C. dematium var. truncatum*, *C. lindemuthianum*, *F. equiseti*, *F. oxysporum*, *F. solani*, *M. phaseolina*, *Myrothecium roridum* and *Phomopsis sojae*. They did not study the pathogenicity of the important seed-borne fungi of soybean. Although a lot of published reports on the seed-borne fungi of soybean are available in different soybean growing countries of the world but the information on the seed-borne fungi and their severity in causing diseases in Bangladesh is scanty. The present study has therefore been undertaken to detect the seed-borne fungi associated with soybean seeds and also to study the pathogenicity of the major seed-borne fungal pathogen.causing serious diseases of soybean.

## MATERIALS AND METHODS

The experiments on the prevalence of seed borne fungi of soybean and the pathogenicity of the major seed-borne fungal pathogens against soybean was conducted in the Microbiology Laboratory of Plant Pathology Department, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur, during September, 2005 to March, 2006.

### *Collection of seed samples*

A total of 33 seed samples of which 3, 16, 4 and 10 were collected from Gazipur (BARI), Mymensing (BAU),

Meherpur (local farmers) and Noakhali (local farmers), respectively during September 2005 to March 2006. Among the collected samples, 15 seed samples were variety Shohag, one variety BARI Soybean 4 and one variety BARI Soybean 5 and rest of the 16 seed samples collected from BAU were genotypes. All the seed samples collected from Meherpur and Noakhali were variety Shohag. The seeds were brought to the Plant Pathology Laboratory of BSMRAU and stored at room temperature for subsequent studies.

***Detection and identification of fungi***

Seed samples were analyzed for the detection of seed-borne fungi by blotter method following International Rules for Seed Health Testing (Anon. 1976) with some modifications. In this method three layers of blotter paper were socked in sterilized water and placed at the bottom of the petridish. 100 seeds taken randomly from each sample and were placed in 10 petridishes (10 seeds per petridish). The petridishes with seeds were then incubated at room temperature for 7 days in the Laboratory. Germination and fungi associated with the seeds were also recorded during the incubation period. Each of the incubated seeds was examined under steriobinoculer microscope to ascertain the presence of fungi. Sometimes fungi were not apparent even after seven days of the incubation. In such condition, the petridishes were allowed for further incubation. A temporary slide was prepared from each fungal colony, which could not be identified under steriobinocular microscope. Fungi were identified by preparing temporary slides and examining under the compound microscope. In fewer cases the fungi from the incubated seeds were transferred to PDA medium in petridishes aseptically and incubated under controlled temperature ($28\pm1^{o}$C) for 3-10 days and then examined under compound microscope. Identification of fungi was confirmed observing their growth character on the slides under compound microscope by using a standard key as described by Rubert and Streets (1982).

***Pathogenicity test of major seed-borne fungi on soybean plants***

Based on the results of the prevalence study, two major fungal pathogens were found to be most predominant and selected for pathogenicity test against soybean plants.

***Colletotrichum dematium***

A total of 25 isolates of *C. dematium* was evaluated for the anthracnose symptom development on the leaf on soybean variety Shohag collected from Noakhali district (N-3). All the isolates were collected from the *C. dematium* infected seed samples analyzed for the prevalence of seed-borne fungi of soybean. Seven days old culture of *C. dematium* grown on PDA was flooded with 10 ml of sterilized distilled water. Acervuli and conidia along with mycelial mass were separated from the substratum by scrapping with a narrow edged sterilized glass slide. The suspension was sieved through double layer cheese cloth to discard acervuli and mycelial mass. The spore suspension was adjusted to $5 \times 10^5$ conidia $ml^{-1}$ by adding sterilized distilled water and counting under stereo binocular microscope using counting slide haemocytometer.

The pathogenicity of *C. dematium* isolates was checked against soybean plants in a pot culture. Each earthen pot was filled with 1.0 kg sterilized soil. Three replicated pots were used for each isolate and 10 soybean seeds were sown in each pot. Inoculation was done when more than 80% seedling reached at 4-5 leaf stage. The inoculum suspension was sprayed in the evening on the soybean seedlings by using a Hand sprayer. The seedlings under control treatment were sprayed only with distilled water. After inoculation seedlings were covered with polythene bags moistened from time to time to ensure proper humidity. Polythene bags were removed from the inoculated seedlings 36 hours after inoculation. Anthracnose symptom development on the detached inoculated leaves was recorded 72 hours after inoculation. Based on the severity of the symptom appearance on the leaf, disease development was graded as ++++, +++, ++, + and – representing highly virulent, virulent, moderately virulent, weak pathogen and avirulent, respectively. Reisolation from the artificially inoculated leaves has been done to ensure the causal pathogen as C. *dematium.*

***Fusarium oxysporum***

A total of 33 isolates of *F. oxysporum* was evaluated for the foot and root rot or wilting symptom development of soybean seedlings against soybean variety Shohag of Meherpur district (M-2). The selected isolates were also collected from the seed samples analyzed for the prevalence of seed-borne fungi of soybean. Wheat grains were used as substrates for the preparation of inoculum of the selected 33 isolates of *F. oxysporum*. Wheat grains soaked in water over night was taken in the 500 ml Erlenmeyer (Pyrex) flasks up to1/3 from the bottom and autoclaved under 1.1 $Kg/cm^2$ pressure at 121 $^{o}$C for an hour. Separate flasks were used for the preparation of each isolate. Ten mycelial discs each of 5 mm diameter were cut from the edge of three days old PDA culture of petrdishes and added into the flasks for each isolate. Flasks containing autoclaved wheat grain with the inoculum were then incubated at 25 $^{o}$C for 20 days. The incubated flasks were shaken by hand at 2-3 days

interval for even colonization. The colonized wheat grains were air dried for 2 weeks and stored at 10 $^{o}$C for further use.

The pathogenicity of the selected 33 isolates of *F. oxysporum* was checked on soybean plants by inoculating pot soil and planting seeds before setting subsequent experiments in a pot culture. Each earthen pot was filled with 1.0 kg sterilized soil. Inocula of each isolate of *F. oxysporum* were thoroughly mixed with sterilized soil @ 20g/kg soil. Controls were prepared using sterilized soil only. Thirty seeds of soybean were sown for each isolate of *F. oxysporum* in three replicated pots. Foot rot and wilting were observed regularly and recorded at 10 and 20 days after planting. Plants infected from 80 to 100% were graded as highly virulent, 70 to 79% infection was graded as virulent, 50 to 69% infection was graded as moderately virulent and less than 50% infection was considered as weak pathogen. Re-isolation of the pathogen from the infected plants was also done to confirm the causal agent of foot rot and wilting. The disease development was expressed as % plant infected.

## RESULTS AND DISCUSSION

### *Prevalence of seed borne fungi of soybean*

Altogether ten fungi comprising nine genera namely *Alternaria alternata*, *Aspergillus flavus, Aspergillus niger, Cheatomium globosum, Colletotrichum dematium*, *Curvularia lunata*, *Fusarium oxysporum*, *Macrophomina phasaelina*, *Penicillium* sp. *and Rhizopus stolonifer* were isolated from soybean seed samples (Table 1 and Figure 1). The fungal colony and the morphology of the isolated fungi are demonstrated in the Figure 2.

Among the isolated fungi *A. flavus* was most prevalent *followed* by *A. niger*, *F. oxysporum*, *C. dematium* and *Penicillium* sp ranged from 10-72%, 4-28%, 4-38%, 0-22%, 0-17%, respectively. The highest incidence of *A. flavus, F. oxysporum*, *A. niger, A. alternata*, *C. dematium, Penicillium* sp. *C. globosum, C. lunata* and *R. stolonifer* and *M. phasaelina* was 72%, 38%, 28%, 26%, 22%, 21%, 11%, 10% and 4%, respectively. These fungi were reported to seed borne in soybean by a number of other workers (Bhuiyan and Fakir 1993, Garcia *et al*. 1991, Gupta *et al.*1993, Anwar *et al.* 1995). In blotter test, the germination of seed samples varied from 16-98 %. The highest germination of 98 % was recorded on seed sample N-10 variety Shohag from Noakhali district where the most pathogenic fungi *A. alternata*, *C. dematium*, *F. oxysporum* and *M. phasaelina* were either absent or present in a fewer number. The lowest germination was recorded in N-4 and also on variety from Noakhali district in which the incidences of these pathogenic fungi and *Aspergillus* spp. were present dominantly. It has been clearly evident that not only the pathogenic fungi but also the presence of the storage fungi like *Aspergillus* spp. caused lower the germination of the seeds. Where the prevalence of fungi was higher, germination percentage of the seed was lower. Similar observation was also reported by other investigators (Anwar *et al.* 1995).

Among the isolated fungi *F. oxysporum*, *C. dematium*, *A. alternata* and *M. phaseolina* were pathogenic on soybean but the other fungi are not reported as pathogenic rather those were reported as storage fungi on soybean (Gupta *et al.*1993, and Anwar *et al.* 1995).The incidence of the pathogenic fungi *F. oxysporum* was observed on all the 33 tested varieties/lines and *C. dematium* was prevalent on 25 varieties/lines and also considered as the major pathogen of soybean. *M. phaseolina* causing charcoal rot of soybean was also a major disease but in this prevalence study the incidence of *M. phasaelina* as a seed-borne pathogen was found very negligible. Based on the prevalence of the pathogen associated with soybean seeds in the current study, *F. oxysporum* and *C. dematium* was selected for pathogenicity tests against soybean plants.

### *Pathogenicity test of C. dematium and F. oxysporum on soybean plants*

The results of pathogenicity test of selected 25 isolates of *C. dematium* against soybean seedlings are presented in the Table 2. The symptoms of anthracnose appeared on soybean plants are shown in the Figure 3. Two isolates namely C19 and C20 collected from Noakhali district of variety Shohag appeared as virulent. Twelve isolates including C4, C5, C8, C10, C12, C15, C17, C18, C21, C22, C23 and C25 were appeared as moderately virulent while rest of the 11 isolates were found to be weak pathogen developing minute and minimum anthracnose spots on the inoculated leaf surface. Among the tested isolates of *F. oxysporum* only three isolates namely F21, F22 and F23 were highly virulent. Isolates F15, F18, F25, F26, F28, F29 and F32 were virulent and the isolates F1, F2, F4, F5, F7, F10, F13, F16, F17, F20, F24, F27 and F31 were moderately virulent and rest of the isolates F3, F6, F8, F9, F11, F12, F14, F19, F30 and F33 were appeared to be weak pathogens (Table 3 and Figure 3). The highest 93.33% plants showed foot and root rot or wilting symptom by the isolate F21, 86.67% by the isolate F22 and 83.33% by the isolate F23 on the variety Shohag M-2, M-3 and M-4 collected from Meherpur, respectively.

In fact, it is difficult to conclude about the degree of virulence as only single method of inoculation has been used and observation was also done for shorter period in pot culture. In the field condition with different methods of inoculation the degree of virulence of the isolates may be varied greatly. The results of the present study of the pathogenicity are in agreement with several investigators who also reported that *C. dematium* and *F. oxysporum* were highly pathogenic causing anthracnose and foot and root rot or wilt diseases of soybean, respectively (Roy 1982, Shchelko *et al.* 1982, Skripka *et al.* 1989 and Johansen *et al.* 1994).

Table 1. Prevalence of different seed-borne fungi associated with soybean seeds

| Seed Samples | % prevalence of fungi | | | | | | | | | | %Germination |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Fusarium oxysporum* | *Alternaria alternata* | *Colletotrichum dematium* | *Curvularia* lunata | *Macrophomina phaseolina* | *Chaetomium* globosum | *Aspergillus flavus* | *Aspergillus niger* | *Penicillium* sp. | *Rhizopus stolonifer* | |
| Shohag | 24 | 4 | 3 | 2 | 0 | 4 | 27 | 12 | 7 | 0 | 63 |
| Soybean-4 | 12 | 3 | 7 | 0 | 0 | 2 | 22 | 13 | 6 | 0 | 70 |
| Soybean-5 | 16 | 2 | 5 | 4 | 0 | 2 | 28 | 7 | 7 | 0 | 80 |
| AGS-120 | 20 | 0 | 9 | 0 | 2 | 0 | 19 | 20 | 11 | 4 | 75 |
| Asset-93 | 10 | 5 | 11 | 0 | 0 | 0 | 12 | 16 | 11 | 3 | 74 |
| G-2261 | 4 | 0 | 0 | 5 | 0 | 3 | 17 | 9 | 13 | 6 | 94 |
| BAU-20 | 26 | 4 | 4 | 2 | 0 | 11 | 23 | 12 | 17 | 5 | 71 |
| BAU-5 | 7 | 0 | 7 | 0 | 0 | 0 | 20 | 15 | 12 | 8 | 87 |
| G-2120 | 4 | 0 | 0 | 0 | 0 | 5 | 30 | 4 | 21 | 4 | 92 |
| ACC-1222 | 20 | 2 | 8 | 3 | 0 | 4 | 20 | 4 | 12 | 10 | 58 |
| AGS-95 | 5 | 0 | 0 | 0 | 0 | 6 | 31 | 14 | 11 | 3 | 90 |
| Gaurab | 8 | 7 | 9 | 0 | 0 | 5 | 27 | 16 | 16 | 9 | 84 |
| MTD | 15 | 2 | 12 | 0 | 0 | 3 | 24 | 14 | 10 | 4 | 59 |
| JS-5 | 4 | 0 | 3 | 0 | 0 | 0 | 10 | 17 | 8 | 7 | 85 |
| Colombus | 20 | 5 | 11 | 0 | 0 | 0 | 33 | 15 | 12 | 0 | 75 |
| G-10180 | 9 | 6 | 4 | 0 | 2 | 0 | 17 | 28 | 13 | 6 | 74 |
| AGS-91 | 11 | 0 | 0 | 3 | 0 | 5 | 16 | 14 | 12 | 2 | 70 |
| BAU-6 | 22 | 0 | 6 | 0 | 0 | 3 | 34 | 28 | 3 | 0 | 81 |
| AGS-302 | 4 | 2 | 9 | 6 | 4 | 0 | 16 | 13 | 17 | 0 | 82 |
| M-1 | 28 | 11 | 0 | 2 | 0 | 0 | 18 | 18 | 13 | 7 | 40 |
| M-2 | 38 | 13 | 10 | 10 | 0 | 0 | 19 | 12 | 10 | 6 | 22 |
| M-3 | 32 | 22 | 0 | 0 | 0 | 0 | 48 | 18 | 3 | 2 | 24 |
| M-4 | 28 | 26 | 11 | 0 | 0 | 0 | 21 | 7 | 2 | 4 | 35 |
| N-1 | 24 | 2 | 0 | 5 | 0 | 2 | 42 | 23 | 5 | 2 | 52 |
| N-2 | 23 | 5 | 7 | 2 | 0 | 6 | 47 | 26 | 2 | 0 | 60 |
| N-3 | 21 | 2 | 22 | 0 | 0 | 2 | 40 | 20 | 0 | 3 | 25 |
| N-4 | 20 | 4 | 16 | 3 | 0 | 0 | 72 | 21 | 0 | 2 | 16 |
| N-5 | 22 | 0 | 10 | 2 | 0 | 3 | 68 | 20 | 0 | 0 | 50 |
| N-6 | 26 | 4 | 12 | 6 | 0 | 2 | 30 | 11 | 7 | 0 | 30 |
| N-7 | 9 | 3 | 15 | 0 | 0 | 0 | 39 | 25 | 4 | 0 | 67 |
| N-8 | 22 | 6 | 8 | 4 | 0 | 0 | 35 | 16 | 5 | 0 | 61 |
| N-9 | 26 | 4 | 16 | 5 | 0 | 0 | 31 | 8 | 2 | 4 | 35 |
| N-10 | 4 | 0 | 0 | 2 | 0 | 0 | 20 | 4 | 0 | 3 | 98 |
| Mean | 17.09 | 4.36 | 7.12 | 2.00 | 0.24 | 2.06 | 28.97 | 15.15 | 8.24 | 3.15 | |

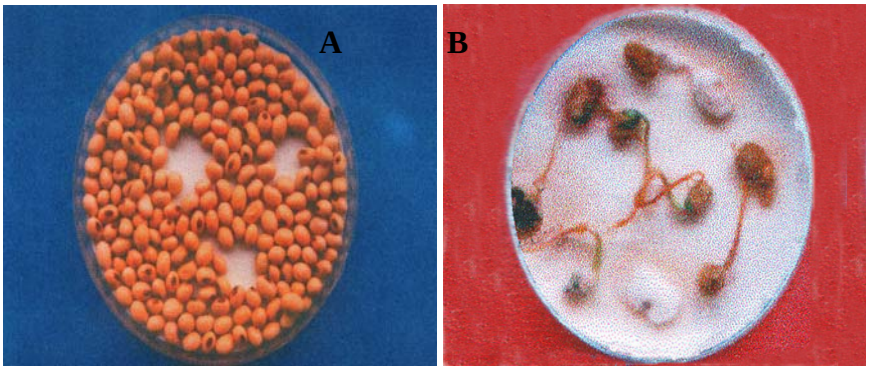


Figure 1. A. Healthy soybean seeds and B. Seed-borne fungi infected soybean seeds seven days after incubation on blotter.

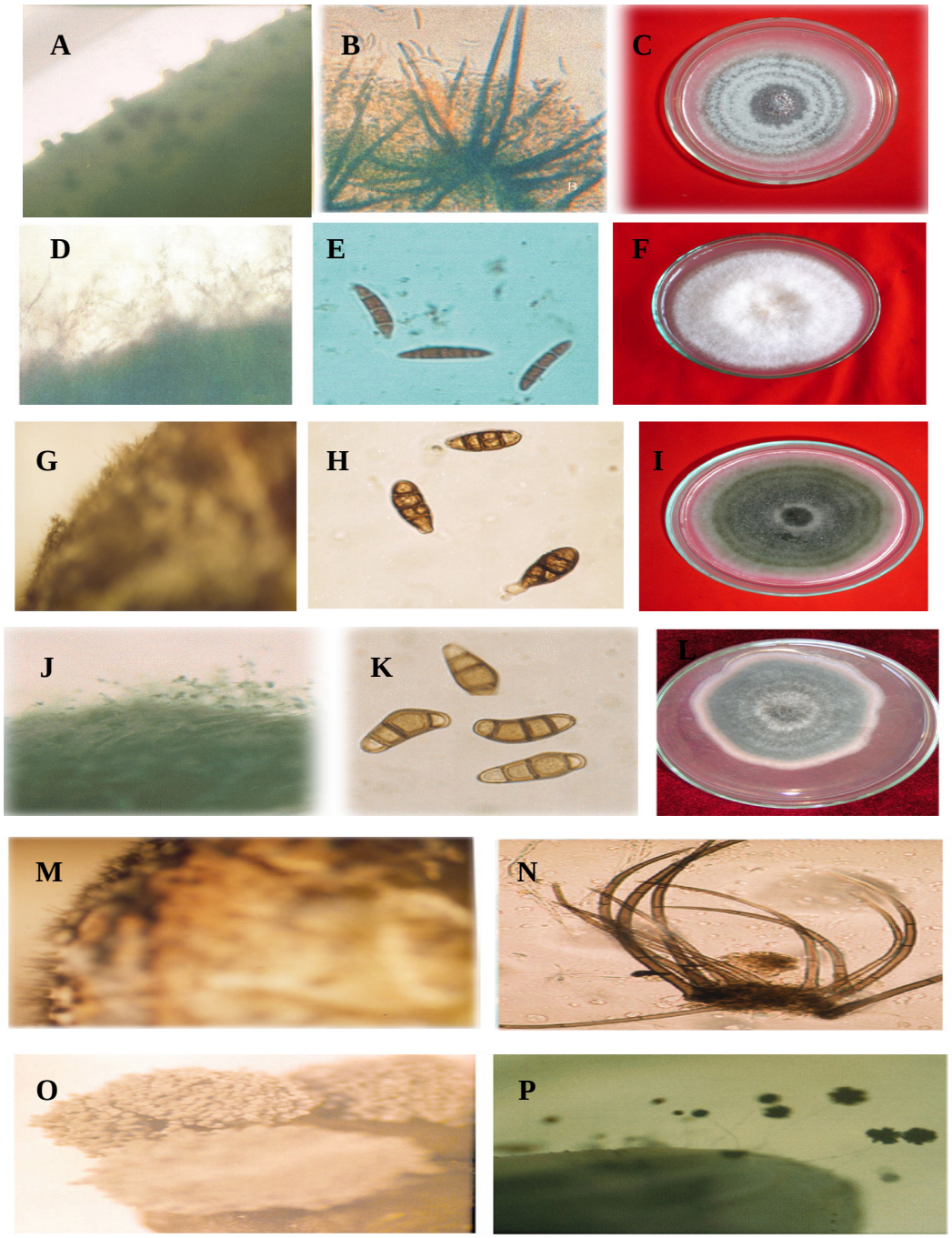


Figure 2. Fungi isolated from soybean seeds [A,B,C *Colletotrichum dematium;* D,E,F *Fusarium oxysporum;* G,H,I *Alternaria alternate* J,K,l *Curvularia lunata;* M,N *Chaetomium globosum* O. *Aspergillus flavus;* P. *Aspergillus niger*.]

Table 2. Pathogenicity test of *Colletotrichum dematium* isolates against soybean vaiety Shohag collected from Noakhali district seed sample N-3 with their origin and locations.

| Isolates of *C. dematium* | Variety/ Genotype | Designation of seed sample | Location | Degree of virulence |
|---|---|---|---|---|
| C1 | Shohag | Shohag | BARI, Joydebpur | + |
| C2 | Soybean-4 | Soybean-4 | BARI, Joydebpur | + |
| C3 | Soybean-5 | Soybean-5 | BARI, Joydebpur | + |
| C4 | AGS-120 | AGS-120 | BAU, Mymensingh | ++ |
| C5 | Asset-93 | Asset-93 | BAU, Mymensingh | ++ |
| C6 | BAU-20 | BAU-20 | BAU, Mymensingh | + |
| C7 | BAU-5 | BAU-5 | BAU, Mymensingh | + |
| C8 | ACC-1222 | ACC-1222 | BAU, Mymensingh | ++ |
| C9 | Gaurab | Gaurab | BAU, Mymensingh | + |
| C10 | MTD | MTD | BAU, Mymensingh | ++ |
| C11 | JS-5 | JS-5 | BAU, Mymensingh | + |
| C12 | Colombus | Colombus | BAU, Mymensingh | ++ |
| C13 | G-10180 | G-10180 | BAU, Mymensingh | + |
| C14 | BAU-6 | BAU-6 | BAU, Mymensingh | + |
| C15 | AGS-302 | AGS-302 | BAU, Mymensingh | ++ |
| C16 | Shohag | M-2 | Meherpur | + |
| C17 | Shohag | M-4 | Meherpur | ++ |
| C18 | Shohag | N-2 | Noakhali | ++ |
| C19 | Shohag | N-3 | Noakhali | +++ |
| C20 | Shohag | N-4 | Noakhali | +++ |
| C21 | Shohag | N-5 | Noakhali | ++ |
| C22 | Shohag | N-6 | Noakhali | ++ |
| C23 | Shohag | N-7 | Noakhali | ++ |
| C24 | Shohag | N-8 | Noakhali | + |
| C25 | Shohag | N-9 | Noakhali | ++ |


Figure 3. Pathogenicity of *C. dematium* and *F. oxysporum* [A: Anthacnose symptoms on soybean leaf; B: *F. oxysporum* infected seedlings; C: Healthy soybean seedling; D: *F. oxysporum* infected soybean seedling's roots; E: Healthy soybean seedling's roots]

Table 3. Pathogenicity test of *Fusarium oxysporum* isolates against soybean vaiety Shohag collected from Meherpur district seed sample M-2 with their origin and locations.

| Isolates of *F. oxysporum* | Variety/Genotype | Designation of seed sample | Location | % plant infected |
|---|---|---|---|---|
| F1 | Shohag | Shohag | BARI | 60.00 |
| F2 | Soybean-4 | Soybean-4 | BARI | 53.33 |
| F3 | Soybean-5 | Soybean-5 | BARI | 46.67 |
| F4 | AGS-120 | AGS-120 | BAU, Mymensingh | 66.67 |
| F5 | Asset- 93 | Asset- 93 | BAU, Mymensingh | 60.00 |
| F6 | G – 2261 | G – 2261 | BAU, Mymensingh | 43.33 |
| F7 | BAU-20 | BAU-20 | BAU, Mymensingh | 63.33 |
| F8 | BAU-5 | BAU-5 | BAU, Mymensingh | 40.00 |
| F9 | G-2120 | G-2120 | BAU, Mymensingh | 33.33 |
| F10 | ACC-1222 | ACC-1222 | BAU, Mymensingh | 66.67 |
| F11 | AGS-95 | AGS-95 | BAU, Mymensingh | 40.00 |
| F12 | Gaurab | Gaurab | BAU, Mymensingh | 33.33 |
| F13 | MTD | MTD | BAU, Mymensingh | 56.67 |
| F14 | JS-5 | JS-5 | BAU, Mymensingh | 33.33 |
| F15 | Colombus | Colombus | BAU, Mymensingh | 70.00 |
| F16 | G-10180 | G-10180 | BAU, Mymensingh | 60.00 |
| F17 | AGS-91 | AGS-91 | BAU, Mymensingh | 60.00 |
| F18 | BAU-6 | BAU-6 | BAU, Mymensingh | 76.67 |
| F19 | AGS-302 | AGS-302 | BAU, Mymensingh | 36.67 |
| F20 | Shohag | M-1 | Meherpur | 63.33 |
| F21 | Shohag | M-2 | Meherpur | 93.33 |
| F22 | Shohag | M-3 | Meherpur | 86.67 |
| F23 | Shohag | M-4 | Meherpur | 83.33 |
| F24 | Shohag | N-1 | Noakhali | 53.33 |
| F25 | Shohag | N-2 | Noakhali | 76.67 |
| F26 | Shohag | N-3 | Noakhali | 73.33 |
| F27 | Shohag | N-4 | Noakhali | 66.67 |
| F28 | Shohag | N-5 | Noakhali | 70.00 |
| F29 | Shohag | N-6 | Noakhali | 73.33 |
| F30 | Shohag | N-7 | Noakhali | 40.00 |
| F31 | Shohag | N-8 | Noakhali | 66.67 |
| F32 | Shohag | N-9 | Noakhali | 70.00 |
| F33 | Shohag | N-10 | Noakhali | 33.33 |

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