

*Article*

## Management of seed borne fungal pathogens of okra collected from seed companies

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**Abstract:** Effect of mehogoni, mehedi and allamanda extracts were tested to control seed borne fungi of okra seeds collected from 6 companies of notunbazar in Mymensingh district. Prevalence of seed borne fungi was studied by blotter method in the Seed Pathology Center (SPC) and MS Laboratory, Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh. The highest germination percentage was recorded from ACI seeds (88%), while lowest (70%) in BADC seeds. Six predominant fungal genera were identified. These species were *Fusarium oxysporum* (5.08%), *Aspergillus flavus* (4.50%), *Aspergillus niger* (6.50%), *Colletotrichum dematium* (4.67%), *Rhizopus stolonifer* (3.33%) and *Penicillium* spp. (3.00%). Germination percentage and fungal association varied from company to company. The germination was ranged from 70-95% and infections were recorded 0.80-6.1% in all the treated seeds. Mehogoni extracts at the rate of 1:1 showed best performance in increasing seed germination (96.00%) next to allamanda (70%). Vigour index of okra seeds were increased 19.14% over untreated seeds by the treatment of mehogoni seed extracts at the rate of 1:1. Mehogoni seed extract at the rate of 1:1 seemed to be adoptable at the farmer's level as an organic management practice.

**Keywords:** FMD; Northern Plateau; seroprevalence; cattle; prevalence odd ratio

### 1. Introduction

Okra (*Abelmoschus esculentus* L.) is a familiar and famous vegetable grown in oriental areas especially in Indian subcontinent. Okra or lady's finger is locally known as "Dherosh" or "Bhendi" which belongs to the family Malvaceae. Owing to their floral morphology and the absence of a self-incompatibility system, they are generally regenerated through selfing. However, depending on the species or variety, season and location, varying degree of outcrossing (up to 6%) occurs in okra. Bees (*Apis mellifera* and *A. cerana*) appear to be the main vectors of pollen (Laboni *et al.*, 2015). It's every 100g green fruits contain 1.8g protein, 6.4g carbohydrate, 1.2g fibre, 18mg vitamin C and 90mg Ca (Rashid, 1999). So people eat and cultivate more okra as compared to other vegetables in Bangladesh. The land area coverage was 25204 acres with production about 42366 metric tons in Bangladesh in 2010-2011 growing season (BBS, 2011). The yield of okra though is not quite high compared to other okra growing countries. Various factors are responsible for low yield of okra. Seed-borne fungal diseases are often the main cause. There are 14 different seed-borne fungal pathogens (Fakir, 2000) causing diseases like seedling blight, stem rot, anthracnose and die-back are considered as major ones. *Aspergillus* spp., *Colletotrichum dematium*, *Fusarium* spp., *Fusarium oxysporum* and *Fusarium moniliforme* are mainly responsible for causing seed-rots (Fakir, 1976). Seed-borne inocula of *Macrophomina phaseolina* and *Colletotrichum dematium* can cause seed rot and seedling blight and the prevalence of both the pathogens depending on the seed sources are 32% and 48%, respectively. *Macrophomina phaseolina* alone can also cause

stem rot, among these fungal pathogens *Colletotrichum dematium* and *Macrophomina phaseolina* are both seed transmitted. Management of these seed-borne fungi is important to produce okra successfully. As there is no known resistant variety, control of these fungi through host resistance is not possible. Again control of these seed-borne fungi using chemicals increase production cost and it is not environmental friendly. Plant extracts had shown good results as seed treating agent. Considerable amount of study have been done with chemical fungicide to control seed-borne disease of okra (Akter, 2008 and Ahmed, 2011). But a few studies were done to control the seed-borne fungi of okra using plant extracts. For these reasons, three plant extracts have been used in this experiment viz. mehogoni extract, mehedi extract and allamanda extracts as seed treating agent. Therefore, there is a great need for recording fungi associated with okra seeds in an easy, quick, reliable and economic seed health testing techniques for proper detection of seed-borne pathogens in the crop. In view of the above facts, the present study was conducted to identify the seed borne fungal pathogen associated with okra seeds and evaluate the efficacy of some botanicals to control the seed borne fungi associated with the okra seeds.

## 2. Materials and Methods

The experiment was conducted at the Seed Pathology Center (SPC) and M.S. Laboratory, Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh. The experiment was conducted during the period from September, 2013 to February, 2014.

### 2.1. Collection of seed samples

A total of six seed samples of okra (*Abelmoschus esculentus* L.) were collected from different seed companies at notunbazar in Mymensingh district. The recommended company's seed was BR-1 variety. The six seed samples were then kept in polythene bags and stored in the refrigerator under 5 °C, till the seeds were used for the subsequent studies.

### 2.2. Dry inspection

The collected seed samples were examined following dry inspection method. The percentage of apparently healthy, diseased, shriveled, discolored and mechanically injured seeds were recorded.

### 2.3. Sprouting test

Germination test was carried out for 400 seeds, drawn randomly from the well-mixed six different companies seeds sample. Ten seeds were plated in each petridish thus 40 petridishes contain 400 seeds. Each petridish was considered as one replication. Three filter papers (Whatman no.1) were soaked in sterile water and placed at the bottom of 9 cm diameter plastic petridish and then 10 seeds were placed on the top of filter paper. The petridishes were placed in incubation room maintaining the temperature at  $20 \pm 2$  °C for ten days. Seeds produced both plumule and radical after incubation were considered as sprouted seeds. No of normal, abnormal and dead seeds were counted separately by this way germination was recorded at 7 days after plating. The result was expressed as percentage.

### 2.4. Detection of seed borne fungi (blotter method)

Seed health status was examined by blotter method. Four hundred seeds were randomly taken from each sample. The seeds were plated on water soaked three layer of whatmanno. 1 filter paper on the plastic petridish. In each petridish, 10 seeds were plated at equal distance. The seeds were incubated at  $20 \pm 2$  °C under 12 hrs. Alternate cycles of Near Ultra Violet (NUV) light and darkness for 7 days. Incubated seeds were examined under stereomicroscope. Seed borne fungi on okra seed surface were detected and identified. Temporary slide was prepared and examined under compound microscope with the help of keys (Ellis, 1971 and Chidambaram *et al*, 1975). Data were recorded on percentage of seed borne fungi.

### 2.5. Efficacy test of plant extracts on the incidence of seed-borne fungi of okra

- a) Mehogoni seed extract at the rate of 1:1, 1:2 and 1:3
- b) Mehedi leaf extract at the rate of 1:1, 1:2 and 1:3
- c) Allamanda extract at the rate of 1:1, 1:2 and 1:3

### 2.5.1. Preparation of plant extracts

Mehogoni, mehedi and allamanda leaf were collected from different areas of Bangladesh Agricultural University, Mymensingh. The collected plant parts were chopped after cleaning under running tap water. The extracts were prepared by crushing the plant parts in a blender with distilled water at 1:1 (100 g crushed plant materials in 100 ml water). One hundred ml water was added to prepare 1:10 dilution. The extracts were filtered through cheese cloth. The extracts thus obtained were kept in a refrigerator at  $4\pm 1^{\circ}\text{C}$  until use. The following treatments were considered for the experiments:

T<sub>0</sub> = Control

T<sub>1</sub> = Mehogoni at the rate of (1:1) w/v

T<sub>2</sub> = Mehogoni at the rate of (1:2) w/v

T<sub>3</sub> = Mehogoni at the rate of (1:3) w/v

T<sub>4</sub> = Mehedi at the rate of (1:1) w/v

T<sub>5</sub> = Mehedi at the rate of (1:2) w/v

T<sub>6</sub> = Mehedi at the rate of (1:3) w/v

T<sub>7</sub> = Allamanda at the rate of (1:1) w/v

T<sub>8</sub> = Allamanda at the rate of (1:2) w/v

T<sub>9</sub> = Allamanda at the rate of (1:3) w/v

### 2.5.2. Seed treatment with plant extracts

Seed samples were treated following dipping method. The seeds were dipped into previously prepared recommended and over dose of mehogoni, mehedi and allamanda extracts suspension as well as 1:1, 1:2 and 1:3 for 30 minutes (Akter, 2008 and Islam, 2009). After proper covering of the seed coat with the extracts the remaining examined plants extracts were drained out from the petridishes. The treated seeds were examined following the standard blotter method. Four replications were maintained for treatment. After incubating the treated seeds, the fungi yielded were observed and germination of seeds was counted.

### 2.6. Vigor test of okra seeds

Four hundred seeds were randomly taken and vigour test was carried out in MS. Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. After 7 days of plating length of shoot was measured from the base of the stem up to the growing point of the youngest leaf. Similarly, length of the root was measured from the starting point of root to the largest available lateral root apex. Shoot and roots were separated from the seedlings. Seedling vigour was determined by the following formula given by Baki and Anderson (1972).

Vigour Index = (Mean of root length + Mean of shoot length) × Seed Germination (%)

### 2.7. Statistical analysis

The collected data were analyzed by analysis of variance. The mean differences among the treatments were compared by completely randomized design (CRD). A statistical computer package MSTAT.C was used for analyzing the data.

## 3. Results

### 3.1. Dry inspection of okra seeds

The results of dry inspection of Okra seeds of 6 companies collected from notunbazar of Mymensingh district are presented in Table 1. Among Six companies apparently healthy seeds were comparatively higher at ACI seeds (93.00 %). Where it was lower at BADC seeds (74.75 %). In dry inspection total seeds were categorized in four which apparently healthy, diseased, shriveled, discolored and mechanically injured seeds by dry inspection. Mechanically injured seeds were found highest at abushama seeds (2.25%) and lowest at ACI seeds (0.75%). Highest shriveled and discolored seeds at local BR-1 (10.75%) and lowest at ACI seeds (1.25%). Highest diseased seeds were found on BADC seeds (17.25%) and lowest at ACI seeds (5.00%) seed and apparently healthy seeds were found highest at ACI seeds (93.00%) and lowest at BADC seeds (74.75 %) of 6 companies collected from notunbazar of Mymensingh district respectively (Table 2).

**Table 1. Particulars of plants used for seed treatment.**

SL. No.	Plants name	Scientific name	Family	Plants parts	Dilution (concentration)
01.	Mehgoni	<i>Swietenia mahagoni</i>	Meliaceae	Seeds	1:1, 1:2 and 1:3
02.	Mehedi	<i>Lawsonia inermis</i>	Lythraceae	Leaf	1:1, 1:2 and 1:3
03.	Allamanda	<i>Allamanda cathartica</i> L.	Apocynaceae	Leaf	1:1, 1:2 and 1:3

**Table 2. Dry inspection of okra seed samples of six companies of notun bazar of Mymensingh district.**

Companies	Apparently Healthy (%)	Diseased (%)	Shriveled and discolored	Mechanically injured (%)
Local BR-1	75.50 d	12.25 d	10.75 a	1.50 c
ACI seeds	93.00 a	5.00 f	1.25 f	0.75 e
Rajib Seeds	90.00 b	5.75 e	3.00 d	1.25 d
Abushama seeds	73.00 f	15.00 c	9.75 b	2.25 a
BADC seeds	74.75 e	17.25 a	6.75 c	1.25 d
Krisan seeds	79.25 c	16.25 b	2.25 e	1.75 b
LSD (0.05)	0.447	0.144	0.233	0.133

### 3.2. Sprouting test of okra seeds

Percentages of seed germination were examined and recorded (Table 3). After 7 days of incubation, the seed samples of okra seeds of 6 companies collected from notunbazar of Mymensingh district showed significant differences in percent germination within a range of 70.00 % to 88.00 % (Table 3). Highest germination was recorded in the ACI seeds (88.00 %). The lowest germination percentage was found in abushama seeds and BADC seeds (70.00 %). Significant differences of germination percentage among the seed samples were found.

**Table 3. Germination percentage of okra seed samples collected from notunbazar of Mymensingh district.**

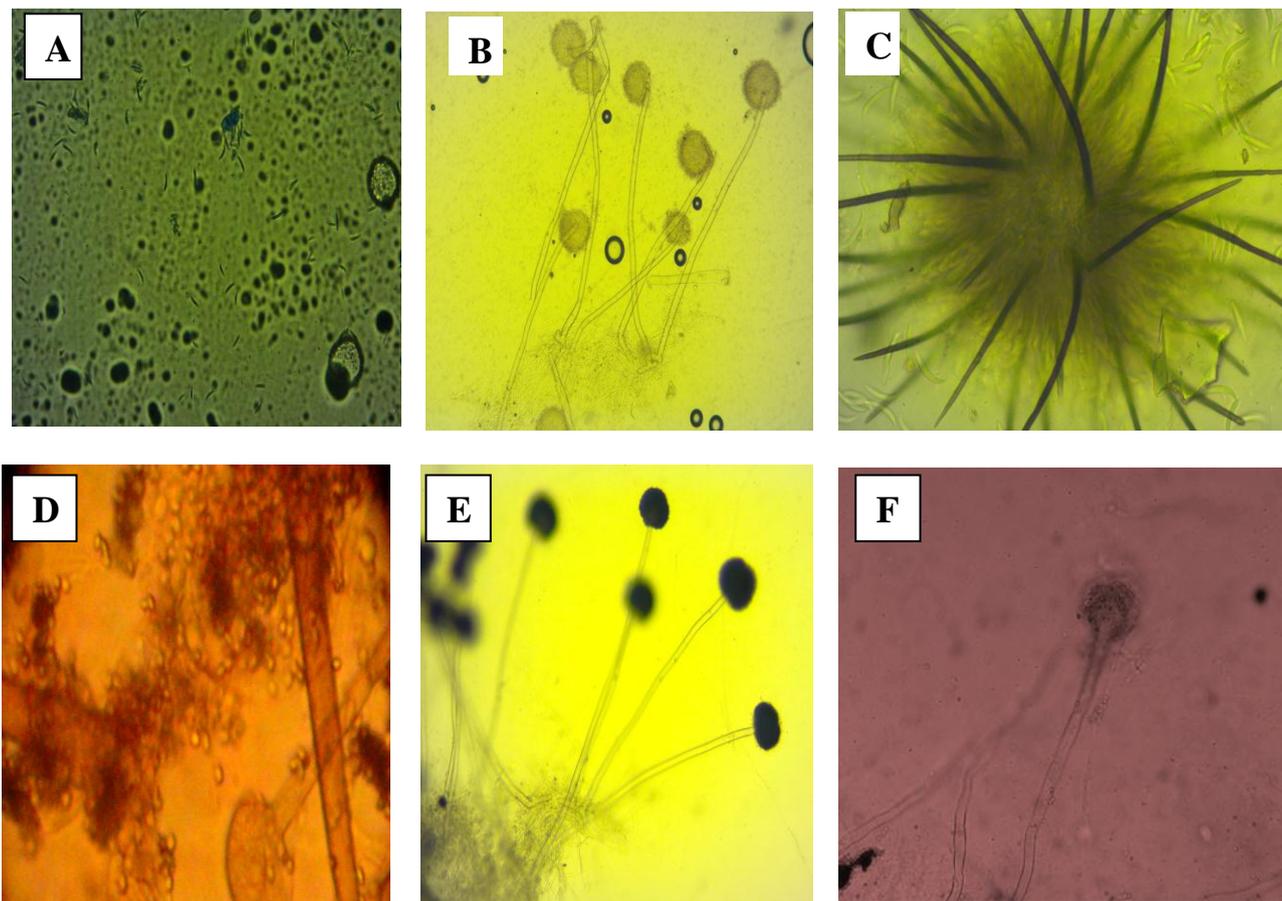
Varieties	Germination (%)
Local BR-1	72 d
ACI seeds	88 a
Rajib Seeds	85 b
Abushama seeds	70 e
BADC seeds	70 e
Krisan seeds	75 c
LSD (0.05)	1.717

### 3.3. Prevalence of fungal genera in okra seeds

Five fungal genera were prevalent in okra seeds during incubation test. The fungal genera were *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, *Colletotrichum dematium*, *Penicillium* spp. and *Rhizopus stolonifer* (Figure 1).

**Table 4. Frequency of occurrence of fungi recorded on Local BR-1 seeds.**

Fungi	No. of fungal infections	% of total infections	No. of infected seeds
<i>Fusarium oxysporum</i>	3.00 e	6.00 b	7.00 a
<i>Aspergillus flavus</i>	4.00 d	4.00 c	6.00 b
<i>Aspergillus niger</i>	3.00 e	2.00 e	6.00 b
<i>Colletotrichum dematium</i>	7.00 a	7.00 a	5.00 c
<i>Rhizopus stolonifer</i>	6.00 b	3.00 d	6.00 b
<i>Penicillium</i> spp.	5.00 c	3.00 d	4.00 d
LSD(0.05)	0.053	0.038	0.026



**Figure 1. Different structures of seed borne pathogens observed under microscope.**

- A. *Fusarium oxysporum* (40×)    B. *Aspergillus flavus* (40×)**  
**C. *Colletotrichum dematium* (40×)    D. *Rhizopus stolonifer* (40×)**  
**E. *Aspergillus niger* (40×)    F. *Penicillium* spp. (40×)**

Association of *Fusarium oxysporum* with 6 companies collected from notunbazar of Mymensingh district was recorded highest in Local BR-1 (8.0%) and lowest in Rajib Seeds (2.0%). Statistically similar association of *Fusarium oxysporum* were observed in abushama seeds (6%), BADC seeds (5.5%), Krisan seeds (6%) and ACI seeds (3.0%) (Table 3). Prevalence of *Aspergillus flavus* with seeds of Notun Bazar in Mymensingh district was found highest in the seed sample in Abushama seeds (10.0%), and lowest in ACI seeds (2.0%). Statistically similar association of *Aspergillus flavus* were observed in Rajib Seeds (4.0%), BADC seeds (4.0%), Local BR-1 (4.0%) and Krisan seeds (3.0 %) (Table 3). The occurrence of *Aspergillus niger* with seeds of Notun Bazar in Mymensingh district was recorded highest in the seed samples collected from BADC seeds (11.0%) and lowest in the sample of ACI seeds (5.0%) and Rajib Seeds (5.0%). While *Aspergillus niger* also showed statistically in Local BR-1 (6.0%), Abushama seeds (6.0%) and Krisan seeds (6.0%). Prevalence of *Colletotrichum dematium* of Notun Bazar in Mymensingh district was recorded highest samples from Abushama seeds (7.0%) and Krisan seeds (7.0%) and lowest in ACI seeds (2.0%). While *Colletotrichum dematium* also showed statistically in Rajib Seeds (3.0%), Local BR-1 (5.0%) BADC seeds (4.0%). Association of *Rhizopus stolonifer* of Notun Bazar in Mymensingh district is highest at Abushama seeds (5.0%) and in lowest position in Rajib Seeds (2.0%) and Local BR-1 (2.0 %) and BADC seeds (2.0%), moreover *Rhizopus stolonifer* prevail at Krisan seeds (6.0%) and ACI seeds (3.0%). Association of *Penicillium* spp. of Notun Bazar in Mymensingh district is highest at Abushama seeds (6.0%) and in lowest position in ACI seeds (2.0%), BADC seeds (2.0%) and Krisan seeds (2.0%) (Tables 4, 5, 6, 7, 8 and 9).

**Table 5. Frequency of occurrence of fungi recorded on Abushama seeds.**

Fungi	No. of fungal infections	% of total infection	No. of infected seeds
<i>Fusarium oxysporum</i>	4.00 d	5.00 c	7.00 a
<i>Aspergillus flavus</i>	9.00 a	7.00 a	6.00 b
<i>Aspergillus niger</i>	3.00 e	4.00 d	6.00 b
<i>Colletotrichum dematium</i>	6.00 b	5.00 c	7.00 a
<i>Rhizopus stolonifer</i>	5.00 c	6.00 b	5.00 c
<i>Penicillium</i> spp.	3.00 e	4.00 d	4.00 d
LSD(0.05)	0.024	0.041	0.082

**Table 6. Frequency of occurrence of fungi recorded on Krisan seeds.**

Fungi	No. of fungal infections	% of total infections	No. of infected seeds
<i>Fusarium oxysporum</i>	3.00 c	4.00 b	2.50 c
<i>Aspergillus flavus</i>	2.00 d	2.00 c	6.50 b
<i>Aspergillus niger</i>	5.00 b	2.50 c	5.50 b
<i>Colletotrichum dematium</i>	7.00 a	5.00 a	7.50 a
<i>Rhizopus stolonifer</i>	3.00 c	2.00 c	1.50 c
<i>Penicillium</i> spp.	2.00 d	1.00 d	1.50 c
LSD(0.05)	0.111	0.204	1.264

**Table 7. Frequency of occurrence of fungi recorded on BADC seeds.**

Fungi	No. of fungal infections	% of total infections	No. of infected seeds
<i>Fusarium oxysporum</i>	4.00 b	3.00 b	5.00 a
<i>Aspergillus flavus</i>	2.00 d	3.00 b	3.00 c
<i>Aspergillus niger</i>	7.00 a	6.00 a	4.00 b
<i>Colletotrichum dematium</i>	3.00 c	2.00 c	4.00 b
<i>Rhizopus stolonifer</i>	2.00 d	1.00 d	2.00 d
<i>Penicillium</i> spp.	3.00 c	2.00 c	1.00 e
LSD(0.05)	0.155	0.162	0.374

**Table 8. Frequency of occurrence of fungi recorded on Razib seeds.**

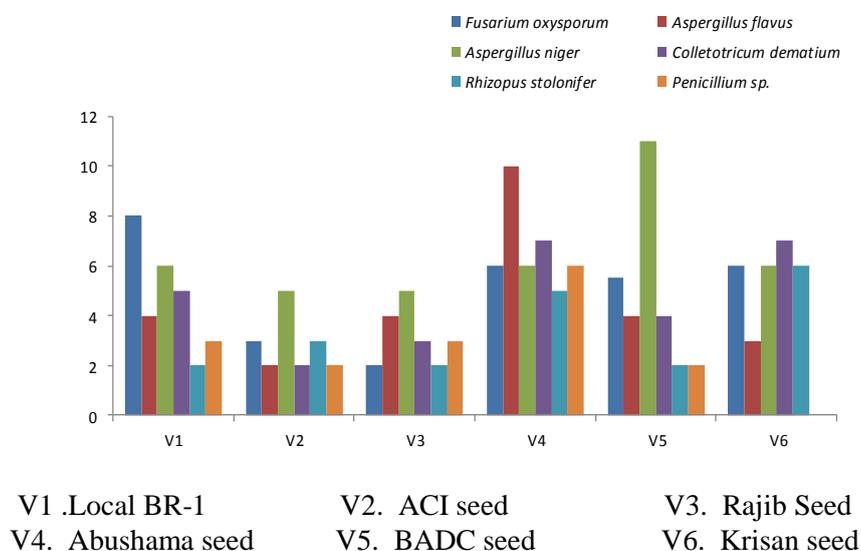
Fungi	No. of fungal infections	% of total infections	No. of infected seeds
<i>Fusarium oxysporum</i>	7.00 a	8.00 a	6.00 a
<i>Aspergillus flavus</i>	2.00 c	3.00 b	5.00 b
<i>Aspergillus niger</i>	1.00 d	2.00 c	3.00 c
<i>Colletotrichum dematium</i>	5.00 b	3.00 b	3.00 c
<i>Rhizopus stolonifer</i>	1.00 d	1.00 d	4.00 c
<i>Penicillium</i> spp.	2.00 c	2.00 c	2.00 d
LSD(0.05)	0.272	0.472	0.240

**Table 9. Frequency of occurrence of fungi recorded on ACI seeds.**

Fungi	No. of fungal infections	% of total infections	No. of infected seeds
<i>Fusarium oxysporum</i>	3.00 b	2.00 d	3.00 a
<i>Aspergillus flavus</i>	1.00 d	3.00 c	2.00 b
<i>Aspergillus niger</i>	3.00 b	5.00 a	3.00 a
<i>Colletotrichum dematium</i>	5.00 a	2.00 d	3.00 a
<i>Rhizopus stolonifer</i>	2.00 c	4.00 b	2.00 b
<i>Penicillium</i> spp.	2.00 c	1.00 e	1.00 c
LSD(0.05)	0.295	0.448	0.234

### 3.4. Effect of plant extracts on seed-borne fungal pathogens of Okra

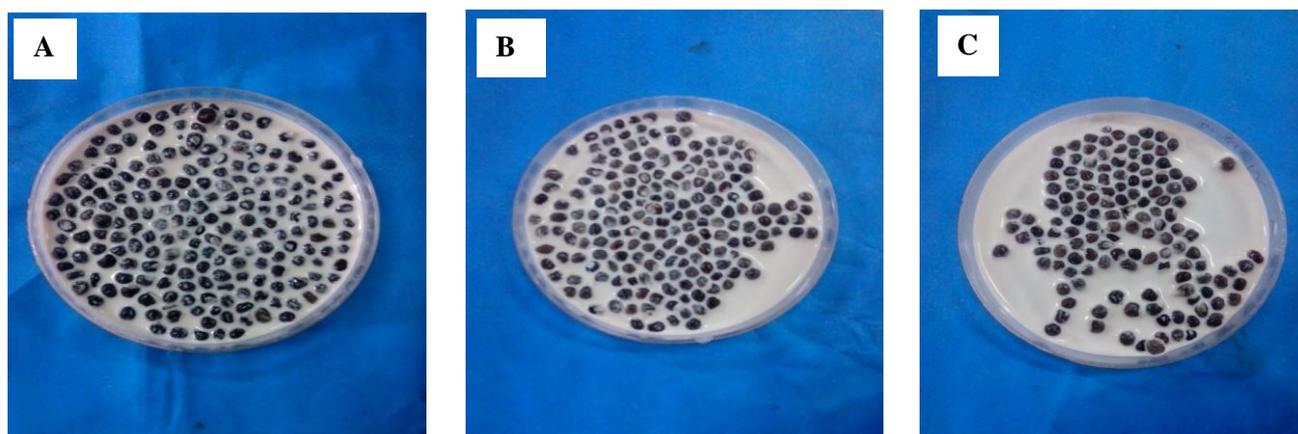
Three plants extracts viz. mehgoni, mehedi as well as allamanda leaf extracts at the rate of (1:1, 1:2 and 1:3) concentrations were used in this experiment. The results are presented in (Tables 10, 11, 12, 13, 14, and 15). Among all the samples collected as different local variety, the seed sample having good performance was subjected to treat with different plant as potential control however Mehgoni extracts is more effective to control the seed borne pathogen. Simultaneously the effectiveness of another botanical extracts is mehedi and allamanda respectively (Figure 2).



**Figure 2. Prevalence of seed borne fungi recorded on 6 different companies of okra seeds collected from Notun Bazar of Mymensingh district.**

### 3.5. Effect of plant extracts on germination of Okra seeds

As germination of the seed is of major concern, it was observed that the treated seeds showed significantly higher rate of germination. From the results, it was also observed that all the extracts increased the percentage of seed germination significantly. Germination was recorded highest (96.00%), when seeds were treated by mehogoniextracts at the rate of 1:1 concentrations in ACI seeds. Comparatively lower percentage of germination was recorded in treatment with allamanda extracts (70.00 %) at the rate of 1:3 concentrations. mehogoni extracts at the rate of 1:1, 1.2 1.3 mehedi extracts at the rate of 1:1, 1.2 and 1:3, allamandaextracts at the rate of 1:1, 1:2 concentrations also gave promising result. In case of control germination is average but pathogen affected (Figure 3, Figure 4, Figure 5).



**Figure 3.(A-C) Seed treated by mehogoniseeds extract 1:1, 1:2 and 1:3 respectively.**



Figure 4.(A-C) Seeds treated by mehedi seeds extract 1:1, 1:2 and 1:3 respectively.

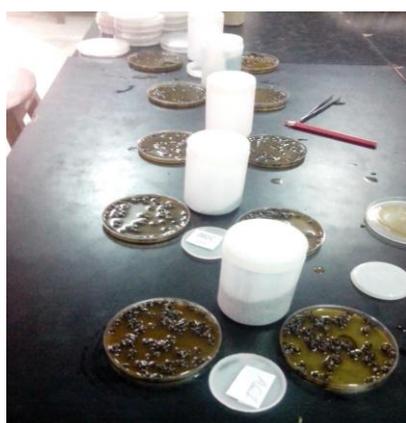


Figure 5. Seeds treated by allamanda leaf extract 1:1, 1:2 and 1:3 respectively.

**3.6. Effect of plant extracts in reducing seed-borne infection of okra seeds**

Seed treatment with mehogni extracts at the rate of 1:1 and 1:2, mehedi 1:1 and 1:2, allamanda extract 1:1, showed excellent performance in controlling *Colletotrichum dematium*. The complete eradication was observed with these treatments. The lower percentage of association of this fungus was also observed with other plant extracts (Tables 10, 11, 12, 13, 14 and 15).

**Table 10. Effect of plant extracts on percent germination and seed borne infection of Okra (BR-1).**

Treatments	% Germination	Percent seed borne infections					<i>Rhizopus stolonifer</i>	<i>Penicillium spp.</i>
		<i>Fusarium oxysporum</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Colletotrichum dematium</i>			
T <sub>0</sub>	72.00 e	3.00 b	4.00 a	3.00 b	7.00 a	6.00 a	5.00 a	
T <sub>1</sub>	80.00 a	1.50 g	2.00 i	1.20 g	2.20 g	2.50 h	2.10 e	
T <sub>2</sub>	77.00 b	1.80 f	2.80 g	2.10 e	2.90 fg	2.80 g	2.30 e	
T <sub>3</sub>	73.00 cd	2.80 c	3.20 d	2.60 d	4.50 d	4.10 cd	2.80 c	
T <sub>4</sub>	78.00 b	1.80 f	2.20 h	1.20 g	3.00 f	2.80 g	2.20 e	
T <sub>5</sub>	74.00 c	2.60 d	2.90 f	2.20 e	3.00 f	3.40 e	2.50 d	
T <sub>6</sub>	71.00 e	3.00 b	3.50 c	2.80 c	4.80 c	4.20 c	2.60 d	
T <sub>7</sub>	77.00 b	2.00 e	2.80 g	1.80 f	2.90 fg	3.00 f	2.20 e	
T <sub>8</sub>	73.00 cd	2.80 c	3.00 e	2.50 d	3.20 e	4.00 d	2.80 c	
T <sub>9</sub>	72.00 de	3.20 a	3.80 b	3.90 a	5.40 b	4.50 b	3.80 b	
LSD(0.05)	1.431	0.088	0.046	0.111	0.188	0.232	0.233	

T<sub>0</sub>: Non-treated (control)

T<sub>1</sub>: Mehogni extract (1:1)

T<sub>2</sub>: Mehogni extract (1:2)

T<sub>3</sub>: mehogni extract (1:3)

T<sub>4</sub>: Mehedi extract (1:1)

T<sub>5</sub>: Mehedi extract (1:2)

T<sub>6</sub>: Mehedi extract (1:3)

T<sub>7</sub>: Allamanda extract (1:1)

T<sub>8</sub>: Allamanda extract (1:2)

T<sub>9</sub>: Allamanda extract (1:3)

**Table 11. Effect of plant extracts on percent germination and seed borne infection of Okra (Abushama).**

Treatments	% Germination	Percent seed borne infections					
		<i>Fusarium oxysporum</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Colletotrichum dematium</i>	<i>Rhizopus stolonifer</i>	<i>Penicillium spp.</i>
T <sub>0</sub>	70.00 f	4.00 a	9.00 a	3.00 c	6.00 a	5.00 b	3.00 b
T <sub>1</sub>	80.00 a	2.00 g	2.50 i	2.10 h	2.20 d	2.50 j	2.20 g
T <sub>2</sub>	78.00 b	2.50 e	3.60 f	2.40 g	3.00 c	3.00 g	2.30 f
T <sub>3</sub>	74.00 d	3.10 c	4.20 c	2.60 f	3.90 b	4.30 e	2.70 c
T <sub>4</sub>	80.00 a	2.20 f	2.80 h	2.40 g	2.40 d	2.80 i	2.20 g
T <sub>5</sub>	76.00 c	2.80 d	3.80 e	2.60 ef	3.20 c	3.10 f	2.40 e
T <sub>6</sub>	72.00 e	3.10 c	4.20 c	2.80 d	4.00 b	4.70 c	2.70 c
T <sub>7</sub>	78.00 b	2.40 e	3.20 g	2.70 e	2.70 cd	2.90 h	2.40 e
T <sub>8</sub>	74.00 d	3.00 c	4.00 d	3.60 b	3.90 b	4.50 d	2.60 d
T <sub>9</sub>	70.00 f	3.50 b	4.60 b	3.90 a	4.40 b	5.60 a	3.20 a
LSD(0.05)	1.218	0.132	0.067	0.088	0.552	0.089	0.091

T<sub>0</sub>: Non-treated (control)T<sub>1</sub>: Mehgoni extract (1:1) T<sub>4</sub>: Mehedi extract (1:1) T<sub>7</sub>: Allamanda extract (1:1)T<sub>2</sub>: Mehgoni extract (1:2) T<sub>5</sub>: Mehedi extract (1:2) T<sub>8</sub>: Allamanda extract (1:2)T<sub>3</sub>: Mehgoni extract (1:3) T<sub>6</sub>: Mehedi extract (1:3) T<sub>9</sub>: Allamanda extract (1:3)**Table 12. Effect of plant extracts on percent germination and seed borne infection of Okra (Krisan).**

Treatments	% Germination	Percent seed borne infections					
		<i>Fusarium oxysporum</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Colletotrichum dematium</i>	<i>Rhizopus stolonifer</i>	<i>Penicillium spp.</i>
T <sub>0</sub>	75.00 d	3.00 a	2.00 ab	5.00 a	7.00 a	3.00 a	2.00 a
T <sub>1</sub>	85.00 a	2.10 e	1.00 f	1.40 g	1.50 g	1.40 ef	0.80 g
T <sub>2</sub>	80.00 bc	2.20 d	1.20 e	1.50 fg	1.90 ef	1.20 f	0.90 g
T <sub>3</sub>	78.00 cd	2.30 c	1.50 d	1.80 e	2.00 def	1.90 c	1.20 de
T <sub>4</sub>	82.00 ab	2.15 de	1.20 e	1.60 f	1.80 f	1.30 ef	0.90 fg
T <sub>5</sub>	80.00 ab	2.30 c	1.30 e	1.90 e	2.10 de	1.50 de	1.10 ef
T <sub>6</sub>	76.00 d	2.50 b	1.60 cd	2.10 d	2.20 d	1.80 c	1.30 d
T <sub>7</sub>	82.00 ab	2.30 c	1.70 c	2.40 c	2.50 c	1.70 cd	1.50 c
T <sub>8</sub>	78.00 cd	3.10 a	1.90 b	2.50 c	2.60 c	1.90 c	1.70 b
T <sub>9</sub>	75.00 d	2.50 b	2.10 a	2.80 b	3.00 b	2.40 b	2.00 a
LSD(0.05)	3.236	0.089	0.173	0.181	0.214	0.263	0.172

T<sub>0</sub>: Non-treated (control)T<sub>1</sub>: Mehgoni extract (1:1) T<sub>4</sub>: Mehedi extract (1:1) T<sub>7</sub>: Allamanda extract (1:1)T<sub>2</sub>: Mehgoni extract (1:2) T<sub>5</sub>: Mehedi extract (1:2) T<sub>8</sub>: Allamanda extract (1:2)T<sub>3</sub>: Mehgoni extract (1:3) T<sub>6</sub>: Mehedi extract (1:3) T<sub>9</sub>: Allamanda extract (1:3)**Table 13. Effect of plant extracts on percent germination and seed borne infection of Okra seeds (BADC).**

Treatments	% Germination	Percent seed borne infections					
		<i>Fusarium oxysporum</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Colletotrichum dematium</i>	<i>Rhizopus stolonifer</i>	<i>Penicillium spp.</i>
T <sub>0</sub>	70.00 f	4.00 a	2.00 b	7.00 a	3.00 a	2.00 a	3.00 a
T <sub>1</sub>	82.00 a	2.00 f	1.60 de	3.20 f	1.60 e	0.50 f	1.20 e
T <sub>2</sub>	80.00 ab	2.10 ef	1.50 e	3.50 ef	1.70 de	0.90 de	1.30 e
T <sub>3</sub>	78.00 bc	2.30 de	1.70 cd	3.90 cd	1.90 c	1.00 d	1.60 cd
T <sub>4</sub>	82.00 a	2.50 d	1.50 e	3.40 f	1.60 c	0.80 e	1.00 f
T <sub>5</sub>	78.00 bc	2.30 de	1.60 de	3.80 de	1.80 cd	1.20 c	1.20 e
T <sub>6</sub>	76.00 cd	2.50 d	1.80 c	4.10 cd	1.80 cd	1.50 b	1.50 d
T <sub>7</sub>	78.00 bc	3.00 c	1.80 c	4.20 c	1.90 e	1.00 d	1.50 d
T <sub>8</sub>	74.00 de	3.60 b	2.00 b	4.60 b	2.20 b	1.20 c	1.70 c
T <sub>9</sub>	72.00 ef	3.65 b	2.50 a	4.90 b	2.35 b	1.50 b	2.10 b
LSD (0.05)	3.404	0.203	0.173	0.310	0.196	0.191	0.173

T<sub>0</sub>: Non-treated (control)T<sub>1</sub>: Mehgoni extract (1:1) T<sub>4</sub>: Mehedi extract (1:1) T<sub>7</sub>: Allamanda extract (1:1)T<sub>2</sub>: Mehgoni extract (1:2) T<sub>5</sub>: Mehedi extract (1:2) T<sub>8</sub>: Allamanda extract (1:2)T<sub>3</sub>: Mehgoni extract (1:3) T<sub>6</sub>: Mehedi extract (1:3) T<sub>9</sub>: Allamanda extract (1:3)

**Table 14. Effect of plant extracts on percent germination and seed borne infection of Okra seeds (Rajib).**

Treatments	% Germination	Percent seed borne infections					
		<i>Fusarium oxysporum</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Colletotrichum dematium</i>	<i>Rhizopus stolonifer</i>	<i>Penicillium spp.</i>
T <sub>0</sub>	85.00 d	7.00 a	2.00 a	1.00 a	5.00 a	1.00 a	2.00 b
T <sub>1</sub>	95.00 a	2.20 e	0.70 f	0.55 d	2.00 f	0.20 f	1.10 f
T <sub>2</sub>	92.00 b	2.25 e	0.75 ef	0.60 cd	2.25 ef	0.25 ef	1.15 f
T <sub>3</sub>	88.00 c	2.40 e	0.75 ef	0.70 bcd	2.40 ef	0.40 de	1.40 e
T <sub>4</sub>	93.00 ab	3.15 d	0.90 e	0.55 d	2.50 ef	0.20 f	1.25 ef
T <sub>5</sub>	91.00 b	3.50 cd	0.80 ef	0.65 bcd	2.55 def	0.30 ef	1.60 d
T <sub>6</sub>	88.00 c	3.60 cd	1.10 d	0.70 bcd	2.80 cde	0.35 def	1.65 cd
T <sub>7</sub>	92.00 b	4.00 c	1.25 cd	0.80 abc	3.10 bcd	0.50 cd	1.60 d
T <sub>8</sub>	88.00 c	4.65 b	1.30 c	0.85 ab	3.25 bc	0.65 bc	2.50 a
T <sub>9</sub>	86.00 cd	5.15 b	1.50 b	1.00 a	3.50 b	0.80 b	1.80 c
LSD(0.05)	2.347	0.565	0.173	0.213	0.575	0.191	0.191

T<sub>0</sub>: Non-treated (control)T<sub>1</sub>: Mehgoni extract (1:1)T<sub>4</sub>: Mehedi extract (1:1)T<sub>7</sub>: Allamanda extract (1:1)T<sub>2</sub>: Mehgoni extract (1:2)T<sub>5</sub>: Mehedi extract (1:2)T<sub>8</sub>: Allamanda extract (1:2)T<sub>3</sub>: Mehgoni extract (1:3)T<sub>6</sub>: Mehedi extract (1:3)T<sub>9</sub>: Allamanda extract (1:3)**Table 15. Effect of plant extracts on percent germination and seed borne infection of Okra seeds (ACI).**

Treatments	% Germination	Percent seed borne infections					
		<i>Fusarium oxysporum</i>	<i>Aspergillus flavus</i>	<i>Aspergillus niger</i>	<i>Colletotrichum dematium</i>	<i>Rhizopus stolonifer</i>	<i>Penicillium spp.</i>
T <sub>0</sub>	88.00 b	3.00 a	1.00 ab	3.00 a	5.00 a	2.00 a	2.00 a
T <sub>1</sub>	96.00 a	1.20 fg	0.50 d	1.00 f	2.00 g	1.20 f	1.10 e
T <sub>2</sub>	94.00 a	1.30 f	0.84 bc	1.20 ef	2.30 fg	1.25 ef	1.15 de
T <sub>3</sub>	90.00 b	1.80 d	0.90 ab	1.50 de	2.60 ef	1.60 bc	1.30 d
T <sub>4</sub>	95.00 a	1.10 g	0.67 cd	1.40 de	2.60 ef	1.40 de	1.20 de
T <sub>5</sub>	94.00 a	1.60 e	0.80 bc	1.60 d	3.00 de	1.45 cd	1.25 de
T <sub>6</sub>	90.00 b	2.10 c	0.90 ab	2.10 c	3.50 cd	1.60 bc	1.30 d
T <sub>7</sub>	94.00 a	1.50 e	0.80 bc	2.00 c	3.50 cd	1.70 b	1.75 c
T <sub>8</sub>	90.00 b	1.60 e	0.84 bc	2.60 b	3.90 bc	1.70 b	1.78 bc
T <sub>9</sub>	88.00 b	2.50 b	0.94 a	3.00 a	4.10 b	1.95 a	1.90 ab
LSD (0.05)	3.404	0.173	0.129	0.310	0.563	0.173	0.150

T<sub>0</sub>: Non-treated (control)T<sub>1</sub>: Mehgoni extract (1:1)T<sub>4</sub>: Mehedi extract (1:1)T<sub>7</sub>: Allamanda extract (1:1)T<sub>2</sub>: Mehgoni extract (1:2)T<sub>5</sub>: Mehedi extract (1:2)T<sub>8</sub>: Allamanda extract (1:2)T<sub>3</sub>: Mehgoni extract (1:3)T<sub>6</sub>: Mehedi extract (1:3)T<sub>9</sub>: Allamanda extract (1:3)

Application of mehogoni extracts completely eradicated the association of *Colletotrichum dematium*, and *Penicillium spp.* Seed treatment with mehogoni extracts at the rate of 1:1 also completely eradicated these three pathogens including *Rhizopus spp.* Association of *Fusarium oxysporum* was found lowest with treatment of mehogoni while lower association of this fungus was recorded with Mehedi leaf extracts at the rate of 1:1, mehogoni extracts at the rate of 1:1 and allamanda at the rate of 1:2 concentrations. The association of *Penicillium spp.* was recorded lowest (0.80%) whereas in control least association (0.5%) of *Aspergillus flavus* was found by the treatment with mehogoni extracts at the rate of 1:1. The association of *Aspergillus niger* was recorded lowest (0.55%) by the treatment with mehogoni and lower association of *Aspergillus niger* was also recorded by the treatment with mehedi leaf extracts at the rate of 1:1. The association of *Rhizopus spp.* was recorded lowest (0.20%) the treatment of seeds with mehogoni extracts at the rate of 1:1 and mehedi leaf extracts at the rate of 1:1. The association of *Colletotrichum dematium* was recorded lowest (1.60%) in the treatment of mehogoni Seed extracts at the rate of 1:1, mehedi leaf extracts at the rate of 1:1, allamanda leaf extracts at the rate of 1:1.

Application of botanical extracts resulted best in controlling total seed-borne infection (Plate 6-11). Seed treatment with mehogoni Seed extracts (at the rate of 1:1 and 1:2), mehedi leaf extracts (at the rate of 1:1 and 1:2) and allamanda leaf extract (at the rate of 1:1 and 1:2) showed promising performance next to control. Total seed-borne infection was lowest in mehogoni Seed extracts at the rate of 1:1, while the second best performance was recorded by the treatment with mehogoni Seed extracts at the rate of 1:2.

### 3.7. Vigour test of okra seeds

Vigour index of Okra seeds were examined for sample of ACI Seeds. Because the sample of ACI Seeds was carried maximum association of fungi having comparatively lower germination. Shoot length of the seedling varied from 5.30 cm to 6.40 cm, the mean shoot length was 5.80 cm and root length varied from 1.50cm to 2.25 cm, the mean root length was 2.00 cm (Table 16).

**Table 16. Vigour test of non-treated and treated okra seeds.**

Sample Name	Germinated seed (%)	Non-germinated seed (%)	Mean shoot length (cm)	Mean root length (cm)	Vigour Index (%)
Nontreated	88.00	12.00	5.60	2.00	668.80
Treated	96.00	4.00	6.1	2.2	796.80

Again, the seed sample of ACI Seeds was treated with mehogni Seeds at the rate of (1:1) concentration and taken for vigour test. Shoot length of the seedling varied from 5.6cm to 7.10 cm, the mean shoot length was 6.10 cm and root length varied from 1.6 cm to 2.50 cm, the mean root length was 2.2cm. The worst performing seed samples were subjected to treat with best performing plant extract to observe 'vigour test' (Table 16). Vigour test was found to be increased considerably after treatment.

## 4. Discussion

Botanical extract (mehogni seed extracts, mehedi and allamanda leaf extracts) has a great effect on treatment with Seed-borne fungi which cause considerable damage to okra crop, so prevention of these fungi is of great importance. The present research program was undertaken to achieve this goal. In this work, the seed-borne fungi associated with okra seeds of companies were investigated as well as some control measures using plant extract were studied. Effect of treatment with plant extract (mehogni seed extracts, mehedi and allamanda leaf extracts) in different dilution ratio on various seed borne pathogens was investigated at Seed Pathology Centre, Bangladesh Agricultural University, Mymensingh this year. To assess seed health condition, dry inspection was done. In dry inspection total seeds were categorized in four groups which apparently healthy, diseased, shriveled and discolored and mechanically injured seeds. Mechanically injured seeds were highest at abushama seeds (2.25%), and lowest at ACI seeds (0.75%) Highest shriveled and discolored seeds were at Local BR-1 (10.75%) and lowest at ACI seeds (1.25%) Highest diseased at BADC seeds (17.25%) and lowest at ACI seeds (5.00%) and apparently healthy highest at ACI seeds (93.00%) and lowest at abushama seeds (73.00%). of notun bazar of Mymensingh district respectively. According to the findings of different workers, it is corroborated that the fungi associated with seed affects the germination of seeds (Richardson, 1990). In the present work, differences were observed in germination percentage among the seed samples. These differences might be due to collection of samples from different Companies, differences in storage condition as well as the quantity and kinds of seed-borne fungal flora associated with them.

The present findings clearly showed that seven different fungi viz. *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, *Colletotrichum dematium*, *Rizopus stolonifer* and *Penicillium* spp. were found to be associated with okra seeds. A considerable number of seed-borne fungal pathogens belonging to the genera of, *Fusarium*, *Aspergillus*, *Colletotrichum*, *Rhizopus* and *Penicillium* have been detected in okra seeds by many researchers (Alam, 2001; Jamadar *et al.*, 2001). The present investigation revealed that *Colletotrichum dematium*, *Macrophomina phaseolina*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp. and *Rhizopus* spp. were associated with the tested seed samples significantly reduced percent germination. Similar results were reported by some earlier workers (Fakir, 2000; Jamandar *et al.*, 2001).

The seed sample of ACI Seed was treated with Mehogni extract at the rate of (1:1) concentration and it showed 70.00% germination and no *Colletotrichum dematium*, and *Penicillium* spp. were observed. Similar results were reported by (Akther, 2008). However, mehogni extract used in controlling seed-borne infection of different crops showed that mehogni extract was a potential agent to control the seed-borne pathogens of different vegetable crops (Hossain, 2001) Treatment of seed samples of ACI Seed with allamanda extracts at the rate of (1:2) showed 65.00% germination and no *Macrophomina phaseolina*, *Penicillium* spp. and *Rhizopus* spp. were observed after treatment. So, far allamanda extracts against seed-borne fungal pathogens of okra were not evaluated. However, allamanda extracts used in controlling seed-borne pathogens of different crops showed good performance (Meah *et al.* 2004; Islam 2005).

Present findings revealed that mehedi leaf extract at the rate of (1:1) showed 68.00% germination and complete reduction *Colletotrichum dematium*, *Macrophomina phaseolina* and *Penicillium* spp. Extracts of mehedi leaf extract at the rate of (1:1) concentration was found moderately effective as seed treating agent which showed 70.00% germination and it reduced *Macrophomina phaseolina* completely. But research related to treatment of okra seeds using extract had not found before this experiment. However, mehedi leaf extract had potential to reduce seed-borne fungi that was shown in case of different crops (Rahaman *et al.*, 1999; Islam, 2005). Mehgoni extract showed best result in germination (67.25%) and it reduces *Colletotrichum dematium*, and *Penicillium* spp. completely. It is also corroborated the result in case of other crops (Wahid *et al.*, 1995; Rahman *et al.*, 1999, Sultana, 2003).

However, among three botanics Mehgoni extract at the rate of 1:1 has performed best in reducing seed-borne prevalence of all the major fungi and eventually increased germination (Rana, 2006) among the treatments T<sub>1</sub> (mehgoni extract at the rate of 1:1) and next effective is T<sub>2</sub> (mehgoni extract at the rate of 1:2) Seed treated with other doses were also found effective. However, seed treatment with plant extracts results in higher germination in different crops including Okra has been reported by Awal (2005) and Rahaman (2006). Therefore, the results of the present finding are in conformity with results of previous workers like Rahaman (2006), Awal (2005), Rahaman *et al.* (1999).

In the present experiment, mehedi leaf extract at the dilution 1:1(w/v) was found the second most effective extract in controlling seed-borne fungi of Okra that controlled *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp. and *Rhizopus stolonifer* etc. Seed treated with other doses were also found effective. Here dose 1:1 w/v of mehedi leaf extract was found to be effective compared to doses 1:2 w/v and 1:3 w/v. This results also supported the findings of Jebunnaher (2004) where inhibition of fungal growth of *Phomopsis vexans* of Eggplant was made Howlader (2003) reported that seed treatment with allamanda leaf extracts effectively increased germination of Eggplant seeds. Allamanda leaf extracts appeared to be the least strong in reducing seed-borne fungi in okra.

## 5. Conclusions

The health condition and germination of Okra seed was significantly influenced by the treatment of plant extracts. A total of 6 samples of okra seeds were collected from 6 companies of notunbazar in Mymensingh district. At first dry inspection were done for studying health status of collected samples. Seed samples collected from ACI seeds showed higher percentage of healthy seeds (93.00%) and lowest was found in abushama seeds (73.00%) All the samples were assayed by Standard Blotter Method. The associated fungi were *Colletotrichum dematium*, *Fusarium oxysporum*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp. and *Rhizopus* spp. After 7 days of incubation, the seed samples of different variety showed a variation in germination percentage (73.00% to 93.00%). The seed samples of ACI seeds showed highest percentage of germinations (93.00%). Therefore, it can be concluded that seed separation with naked eye will help to get more apparently healthy seeds. It is also revealed from this experiment that mehogoni Seed extracts, mehedi leaf extracts or allamanda leaf extracts can be recommended for okra seed treatment for getting higher germination and healthy seedling that will eventually increase Okra production.

## Conflict of interest

None to declare.

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