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Prevalence of seed borne pathogens of ornamental plants grown in Bangladesh

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ABSTRACT

We investigate the prevalence of pathogens associated with seeds of some selected local flowers namely cosmos, marigold, and silvia and corms of gladiolus collected from three different locations (Jessore, Savar and Gazipur) were recorded. Germination test for the collected seeds were performed and pathogens were isolated and identified. The seeds from Jessore were better than the others considering incidence of fungi in all other seeds tested. Silvia from Jessore was the lowest affected seed and marigold from savar was the highest affected seed. *Fusarium* sp., *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer*, *Alternaria alternate*, *Penicillium*, *Curvularia*, *Chaetomium* and *Cladosporium* sp. comprising 9 species were found to be associated with the flower seed sample. The percent of seed borne infection ranged 4-28%, 5-32.5%, 3-29% and 11- 25% in case of cosmos, marigold, silvia and gladious, respectively. Silvia was the lowest affected seed (13.5, 18.42 and 8.42 average incidence) and marigold was the highest affected seed (12.18, 19.25 and 11.5% average incidence) collected from Gazipur, Savar, and Jessore, respectively. The result showed that the highest percentage germination of cosmos (86.5%), marigold (77.5%), silvia (54.5%) obtained from Jessore and gladiolus (80%) obtained from Gazipur. So, the seeds collected from Jessore were best germination and seed health quality and can be suggest for using the commercial cultivation of ornamental plants.

INTRODUCTION

Seed-borne diseases affect a wide range of ornamental crops and potentially may result in substantial and widespread crop losses, disruption to production schedules and increased use of plant protection products. Ignorance of the importance of flower seed-borne diseases is often common. Plant diseases may be found in, on or with seed and seed lots. Seed-borne diseases may be grouped according to their causal agents, fungi, bacteria, virus, nematodes etc. (Sheppard, 1998). According to a recent market report from Market Research, the global flower market was valued around US$ 67.3 billion in 2017 and is projected to reach a value of US$ 103.9 billion by 2026, registering a CAGR of 5% during the forecast period. Approximately, 10,000 hectare of land is now under flower cultivation in Bangladesh. At present, there are 2000 flower shops in the country and flowers worth Tk. 100 crores are sold annually (Noor, 2010). According to an Article in Team India, Flower farming is nearly 5% more profitable than rice cultivation, 2% more profitable than growing vegetables. By exporting flowers, our country can also earn a good amount of foreign exchange. Thus, there is a great prospect of flower cultivation in Bangladesh.

Flower plants suffer from more than 100 diseases according to U.S. Department of Agriculture (USDA, 1960). Of these, 12 diseases caused by 20 fungal pathogens, recorded on the eight selected flower plants cosmos, periwinkle, marigold and calendula are known to be local seed-borne (Richardson, 1990). Flower plants suffer from more than 100 diseases according to U.S. Department of Agriculture (USDA, 1960). Of these, 12 diseases caused by 20 fungal pathogens, recorded on the eight selected flower plants cosmos, periwinkle, marigold and calendula are known to be local seed-borne (Richardson, 1990).

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Sultana (2004) recorded six seed borne fungi on cosmos seeds. All these seed-borne pathogens are responsible for causing diseases in the respective flower plants mentioned and incur losses to flower production. Javaid et al. (2008) found that *Fusarium oxysporum* f. sp *gladioli* (Massey) Snyd. & Hans. from diseased corms of gladiolus *(Gladiolus grandiflorus* sect. Blandus) cv. Aarti.

The true impact of seed borne pathogens may be greater than is commonly appreciated due to the uncertain origin of many disease outbreaks and the potential for latency in specific pathogen groups. There has been much less investigation into the seed pathology of flower crops compared with field crops and vegetables (Richardson, 1979). As flower crops may share some common pathogens with other crops, it is necessary to investigate the seed-borne pathogens of commonly grown flower crops. Effective disease management programs and the development of reliable and accurate methods for seed health testing are dependent upon an understanding of plant disease epidemiology. Thus, the study was undertaken to provide a comprehensive listing of seed-borne diseases of protected ornamental crops commonly grown in the sub-continent, to determine the current prevalence of fungi and bacteria on some commercial seed lots.

MATERIALS AND METHOD

Seeds collections and maintenance

Seeds of some selected local flower namely cosmos, marigold, silvia, and corms of gladiolus were collected during 2016 and 2017 from three different locations (Jessore, Savar and Gazipur). The samples were stored at 5 °C until use. The experiments were conducted in Mycology, Plant Pathology division in Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh.

Germination test

This experiment was done by using standard Paper Towel Method (ISTA, 2003). Germination rate was calculated as a ratio between the total number of germinated seeds and the total number of experimental seeds was expressed in percent.

Isolation and identification of seed-borne pathogens

Direct isolation method was performed for isolation of fungal pathogens from the selected seeds. This method consisted of incubation of seeds in humid chamber with surface sterilization following Standard Blotter Method (ISTA, 2003). Each individual incubated seed was observed under Stereo-microscope at 16x and 25x magnifications in order to record the incidence of seed borne fungi. Identification of isolated fungi was done following the Manuals- Dematiaceous Hypomycetes (Ellis, 1971) and illustrated genera of imperfect fungi (Barnett and Hunter, 1998).

RESULTS

Seed health test of cosmos flower

From the total population of fungi encountered in this study, 8 genera *Fusarium* sp., *Aspergilus flavus*, *Aspergilus niger*, *Rhizopus stolonifer*, *Alternaria alternata*, *Penicillium*, *Curvularia*, and *Cladosporium* sp. were identified from the cosmos seeds of selected locations (Figure 1). Among the identified pathogenic fungi all fungi were found in seeds collected from all locations except *Cladosporium* and *Rhizopussto lonifer*. Among these two fungi *Cladosporium* were absent in seeds collected from Jessore and *Rhizopus stolonifer* were absent in Gazipur. The maximum mean percent incidence of seed borne fungi were recorded as 22.67 % for *Fusarium* sp, followed by *Alternaria alternate* (22.50 %), *Penicillium* spp. (14.33%), *Cladosporium* (11.67%), *Chaetomium* (8.00 %), *A. flavus* (6.67%) and *A. niger* (5.83%) The highest and lowest average incidence of fungi recorded as 13.69% and 9.69% for Savar and Jessore, respectively (Table 1).

Table 1: Prevalence of seed borne fungi on cosmos seeds

No.	Organisms	Occurrence of pathogen (%)			Mean % incidence of fungi
		Gazipur	Savar	Jessore	
1.	*Alternaria alternata*	20.5	26.5	20.5	22.50
2.	*Aspergillus flavus*	4.5	9.5	6	6.67
3.	*Aspergillus niger*	4	8	5.5	5.83
4.	*Cladosporium* sp.	16.5	18.5	nd	11.67
5.	*Fusarium* sp.	28	22	18	22.67
6.	*Penicillium* sp.	16.5	10.5	16	14.33
7.	*Chaetomium*	7.5	9	7.5	8.00
8.	*Rhizopus stolonifer*	nd	5.5	4	3.17
	Average	12.19	13.69	9.69	

*nd = not detectable

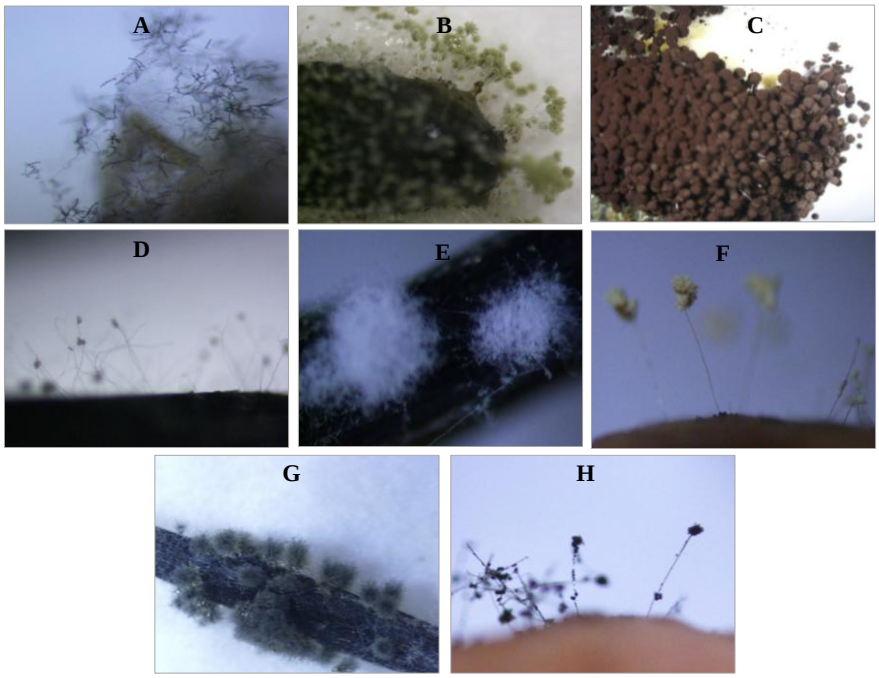

Figure 1: Stereo microscopic view of pathogens on cosmos seeds, (A) *Alternaria alternata,* (B) *Aspergillus flavus,* (C) *Aspergillus niger*, (D) *Cladosporium* sp., (E) *Fusarium* sp., (F) *Peniciliium* sp., (G) *Chaetomium* sp., (H) *Rhizopus stolonifer*

Seed health test of marigold flower

Total 8 genera, *Fusarium* sp., *Aspergilus flavus*, *Aspergilus niger*, *Rhizopus stolonifer*, *Alternaria alternata*, *Penicillium*, *Curvularia*, and *Cladosporium* sp. were identified from the marigold seeds of selected locations (Table 2, Figure 2). In Gazipur, among the identified pathogenic fungi *Curvularia* sp. were found highest percentage (25.5%) of occurrence and *Penicillium* sp. were found lowest percentage (5%) of occurrence. In Savar, the highest percentage of occurrence was *A. flavus* (32.5%) and lowest was *A. niger* (15%). In Jessore, the highest percentage of occurrence was *A. flavus* (25%) and lowest was *Curvularia* (6.5%). The maximum mean percent incidence of seed borne fungi were recorded as 27.17 % for *A. flavus*, followed by *Alternaria alternate* (20.33%), *Curvularia* sp. (19.67 %), *R. stolonifer* (15.25%), *Cladosporium* (15%),

Fusarium sp. (14.83%), *A. niger* (13.50%) and *Penicillium* spp. (10%). The incidence of fungi recorded as 19.25%, 12.18% and 11.5% for Savar, Gazipur and Jessore, respectively.

Table 2: Prevalence of seed borne fungi of marigold flower

No.	Organisms	Occurrence of pathogen (%)			Mean % incidence of fungi
		Gazipur	Savar	Jessore	
1.	Alternaria alternata	18.5	25.5	17	20.33
2.	Aspergillus flavus	24	32.5	25	27.17
3.	Aspergillus niger	nd	15	12	13.50
4.	Cladosporium sp.	15	nd	nd	15.00
5.	Fusarium sp.	14.5	18	12	14.83
6.	Penicillium sp.	5	17	8	10.00
7.	Curvularia	25.5	27	6.5	19.67
8.	Rhizopus stolonifer	nd	19	11.5	15.25
	Average	12.81	19.25	11.5	

*nd = not detectable

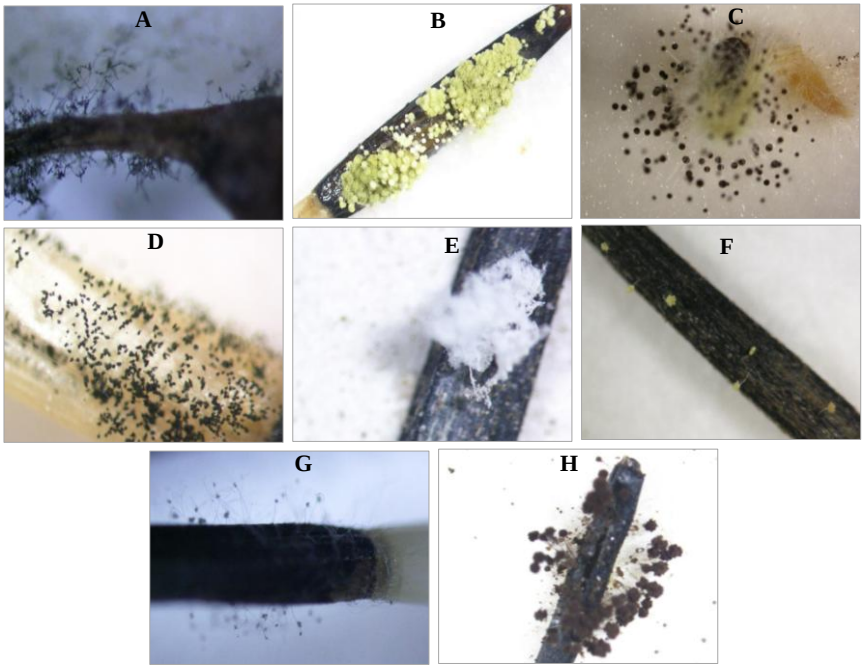

Figure 2: Stereo microscopic view of pathogens on marigold seeds, (A) *Alternaria alternata*, (B) *Aspergillus flavus*, (C) *Aspergillus niger*, (D) *Cladosporium* sp., (E) *Fusarium* sp., (F) *Peniciliium* sp., (G) *Curvularia* sp., (H) *Rhizopus stolonifer*

Seed health test of silvia flower

Total 6 genera, *Fusarium* sp., *Aspergilus flavus*, *Alternaria alternata*, *Penicillium*, *Curvularia*, and *Cladosporium* sp. were identified from Silvia seeds of selected locations (Gazipur, Savar and Jessore) (Table 3, Figure 3). In Gazipur, 23.5% *Penicillium* sp. was found in total examined seeds of silvia flower which was highest in comparison with the rest of the seed-borne pathogens that was found in silvia seeds of Gazipur and *Cladosporium* sp. was occurred as 6% which was lowest. In Savar, the highest percentage of occurrence was *Alternaria*

alternata (29%) and lowest was *Cladosporium* sp. (10%). In Jessore, the highest percentage of occurrence was *Alternaria alternate* (16%) and lowest was *Fusarium* sp. (3%). The maximum mean percent incidence of seed borne fungi were recorded as 21% for *Alternaria alternata*, followed by *Penicillium* sp. (17.5%), *Aspergillus flavus* (14.33%), *Curvularia* sp. (12.50 %), *Fusarium* sp.(10%) and *Cladosporium* (8%). The average incidence of fungi recorded as 13.5%, 18.42% and 8.42% for Gazipur, Savar and Jessore, respectively (Table 3).

Table 3: Prevalence of seed borne fungi of silvia

No.	Organisms	Occurrence of pathogen (%)			Mean (%) incidence of fungi
		Gazipur	Savar	Jessore	
1.	Alternaria alternata	18	29	16	21.00
2.	Aspergillus flavus	13.5	21	8.5	14.33
3.	Fusarium sp.	11.5	15.5	3	10.00
4.	Penicillium sp.	23.5	18.5	10.5	17.50
5.	Curvularia	8.5	16.5	12.5	12.50
6.	Cladosporium sp.	6	10	nd	8.00
	Average	13.5	18.42	8.42	

*nd = not detectable

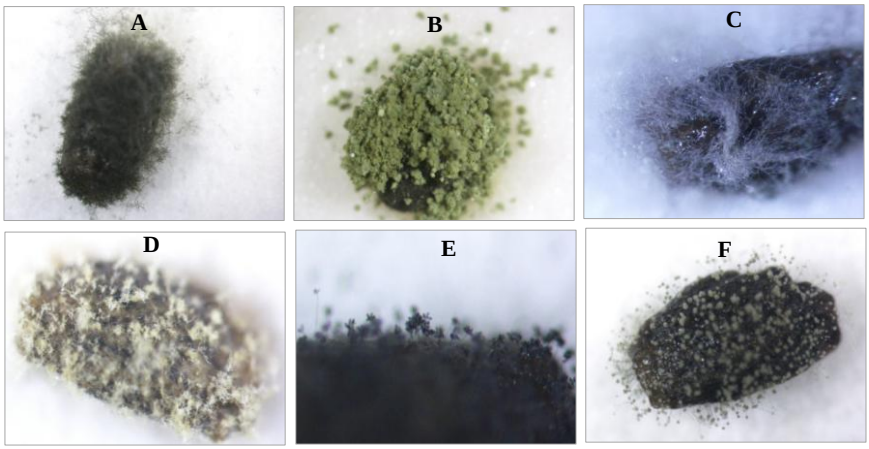

Figure 3: Stereo microscopic view of pathogens on silvia seeds, (A) *Alternaria alternata*, (B) *Aspergillus flavus*, (C) *Fusarium* sp., (D) *Peniciliium* sp., (E) *Curvularia* sp., (F) *Cladosporium* sp.

Seed health test of gladiolus flower

Total 5 genera, *Fusarium* sp., *Aspergilus flavus*, *Aspergillus niger*, *Rhizopus stolonifer* and *Penicillium* were identified from Silvia seeds of selected locations (Gazipur, Savar and Jessore) (Table 4, Figure 4). In Gazipur, the highest occurrence of fungi in the corm of gladiolus was found as *Aspergilus niger* which was 29%, and *Fusarium* sp. was occurred as 11% which was lowest. In Savar, the highest percentage of occurrence was *Aspergilus flavus* (40%) and lowest was *Rhizopus stolonifer* (20%). In Jessore, the highest percentage of occurrence was *Aspergilus flavus* (23.05%) and lowest was *Rhizopus stolonifer* (18%). The maximum mean percent incidence of seed borne fungi were recorded as 29.67% for *Aspergilus flavus*, followed by *Aspergilus niger* (24.5%), *Penicillium sp.* (23.67%), *Rhizopus stolonifer* (19%) and *Fusarium* sp.(17.33 %). The average incidence of fungi was recorded as 20.8 %, 27.5 % and 20.2% for Gazipur, Savar, and Jessore, respectively.

Table 4: Prevalence of seed borne fungi of gladiolus

No.	Organisms	Occurrence of pathogen (%)			Mean % incidence of fungi
		Gazipur	Savar	Jessore	
1.	Fusarium sp.	11	21.5	19.5	17.33
2.	Aspergillus flavus	25.5	40	23.5	29.67
3.	Aspergillus niger	29	25	19.5	24.50
4.	Rhizopus stolonifer	19	20	18	19.00
5.	Penicillium sp.	19.5	31	20.5	23.67
	Average	20.8	27.5	20.2	

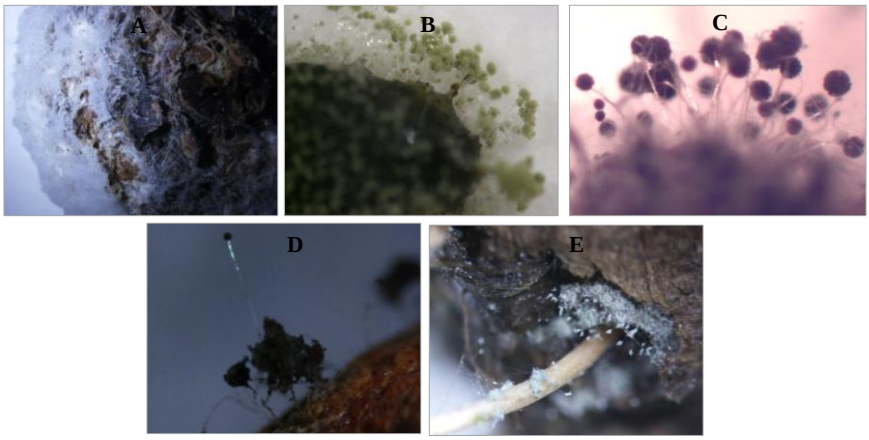

Figure 4: Stereo microscopic view of pathogens on gladiolus seeds, (A) *Fusarium* sp., (B) *Aspergillus flavus*, (C) *Aspergillus niger*, (D) *Rhizopus stolonifer* (E) *Peniciliium* sp.

Germination rate of flower seeds and corms

Flower seed samples of cosmos, marigold, silvia and gladiolus corm were subjected to germination test in which the highest germination of cosmos (86.5%), marigold (77.5%), silvia (54.5%) obtained from Jessore and gladiolus (80%) obtained from Gazipur (Table 5). On the other hand, the lowest germination of cosmos (78.5%), marigold (62%) and gladiolus (60%) obtained from Savar and silvia (40%) from Gazipur.

Table 5: Germination of flower seeds and corms collected from three different sources

SI. No.	Flower name	Seed sources	% Seed germination
1.	Cosmos	Jessore	86.5
		Savar	78.5
		Gazipur	82
2.	Marigold	Jessore	77.5
		Savar	62
		Gazipur	75.5
3.	Silvia	Jessore	54.5
		Savar	47.5
		Gazipur	40
4.	Gladiolus	Jessore	75
		Savar	60
		Gazipur	80

DISCUSSION

The experiment was conducted with three different flower seeds and corm of gladiolus flower was collected from three different flower growing locations (Jessore, Savar and Dhaka). The total population of fungi encountered in this study, 9 genera were identified *Fusarium* sp., *Aspergilus flavus*, *Aspergilus niger*, *Rhizopus stolonifer*, *Alternaria alternata*, *Penicillium*, *Curvularia*, *Chaetomium* and *Cladosporium* sp. were more common. Most of these genera of fungi are common in all flower seeds. These findings

corroborate by Wu et al. (2006), who found *Alternaria*, *Curvularia* and *Drechslera* provided more diversified species than other genera among the flower seeds tested.

The result obtained from seed health test by blotter method revealed that the percent of seed borne infection ranged 4-28%, 5- 32.5%, 3-29% and 11-25% in case of cosmos, marigold silvia and gladious, respectively according to different locations (Jessore, Savar and Dhaka). Again, it was observed that fungal infections were higher in cosmos and marigold seeds compared to silvia and marigold considering all three sources of seeds. The result indicate that the percent of total seed borne infection varied with different locations, but the total occurrence of pathogens were highest in all seeds obtained from Savar and lowest was seeds from Jessore. The prevalence of the total and the individual fungal infection varied with respect of flower spices and sources of seed collection. Such variation in the occurrence of seed-borne fungi has been demonstrated in a number of crops like rice, kaon, mustard, black gram, wheat, jute and chilli by different research workers (Hossain et al. 1977, Barma and Fakir, 1981 and Fakir and Halder, 1993).

The findings of the present study corroborate with the finding of Sultana (2004). She tested calendula, cosmos, marigold and zinnia seeds collected from different sources for prevalence of fungi associated with the seeds. She recorded five seed-borne fungi on calendula seeds. Among the fungi *Alternaria tenuis* was the most prevalent on calendula seeds which support the present study. She recorded six seed borne fungi on cosmos seeds that are in accordance with the present study. In the present study prevalence of all the fungi varied significantly with respect to flower species and seed sources. Twenty-four fungi were detected from 22 seed samples of 13 species of flower crops. Among these fungi, *Alternaria carthami* isolated from zinnia seed, *Colletotrichum dematium* isolated from seeds of celosia and globe amaranth, *Curvularia lunata* isolated from French marigold and globe amaranth, *Drechslera rostrata* isolated from French marigold, and *Phoma* sp. isolated from globe amaranth decreased the rate of germination of their related flower crops significantly. In addition, *A. carthami* and *C. dematium* caused disease to zinnia and celosia, respectively reported by Chou and Wu (1995).They also reported *Alternaria zinnae* on Zinnia (*Zinnia elegans* L.) and other flowers (Wu and Chou, 1995). Orlicz-Luthart (1998) observed *Fusarium oxysporum* f.sp. *callistephi*, on China aster (*Callistephus chinensis* (L.) Nees.).

Germination of different flowers collected from different locations were determined by blotter method were varied significantly according to sources of seeds. Result showed that all the seeds from Savar gave lowest germination rate except silvia which gave lowest germination rate in seeds from Gazipur. Occurrence of pathogens might be reason for lowest germination rate of the seeds obtained from Savar. The result also supported with Shroti et al. (1985), who obtained 20 pathogenic fungi in calendula seeds and found significant reductions in seed germination due to the presence of fungi with the seeds. Seed germination also varied significantly depending on the seed source and flower species which corroborates with the findings of Sultana (2004). As the study was limited in few locations only, further studies with more representative seed samples from different flower species, obtained from different parts of the country should be undertaken in order to portray the exact picture regarding the prevalence of fungi, specially the pathogenic ones in flower seeds.

CONCLUSION

We worked with three different flower seeds *viz.* cosmos, marigold, silvia and corms of gladiolus was collected from three different locations (Jessore, Savar and Gazipur) but in respect of locations seed-borne fungal incidence of Jessore's seed was better than the others, followed by seeds from Gazipur. On the other hand highest percentage of pathogens occurs in the seeds from Savar. But in respect of flower's, silvia seed infection was the lowest and seeds of marigold were the highest infected seed. Percent seed germination by blotter method varied significantly for the flower variety collected from different locations. The risk of introducing a seed-borne disease onto a nursery can be greatly reduced by use of good quality seed from reputable suppliers. Where required, further assurance on the health of

a seed batch with regard to specified pathogens can be obtained by submitting a sample to a diagnostic plant pathology laboratory where tests for specific fungi, bacteria, viruses and viroids can also be done.

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