

Prevalence of Seed-Borne Fungi Associated with Seeds of Some Selected Flowers

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Abstract

The prevalence of fungi associated with seeds of some selected local flowers namely cosmos, marigold, calendula, and periwinkle collected from three different locations (Arboriculture Garden, Ramna Park Garden, and Horticulture Garden) in Dhaka district and four hybrid flower seeds (African marigold, cockscomb, petunia, and *Portulaca*) collected from Momin Beej Ghar, Dhaka district were recorded. The results showed that the highest percent germination (91.38%) of cosmos was obtained from Horticulture garden's seed, marigold (95.25%) obtained from Arboriculture garden's seed, and calendula (65.88%) periwinkle (79.38%) obtained from Ramna park garden's seeds. But in the case of hybrid seeds, African marigold showed the highest percent seed germination (98.25%). The Horticulture garden's seed was better than the others considering the incidence of fungi in all local flower seeds tested. But periwinkle was the lowest affected seed (12.58%, 12.20%, 8.07% incidence) and marigold was the highest affected seed (40.31%, 35.33%, 31.33% incidence) collected from Arboriculture Garden, Ramna Park, and Horticulture Garden, respectively. But in hybrid flower seeds, the lowest fungal infection (3.14%) was recorded in *Portulaca* and petunia seeds whereas the highest (6.20%) was in African marigold seeds.

Keywords

Flower Seed, Seed-Borne Fungi, Dry Inspection, Blotter, Agar Plate

1. Introduction

Seed is the most important input for crop production. In modern agriculture, seed health is a well-recognized factor for increased production. Pathogen-free healthy seeds are considered a vital input for desired plant populations and a good harvest. Seed health testing is done to determine microbial infection or

contamination for quarantine purposes. It identifies the cause of seed infection that affects the planting value of seed lots for seed certification by seed growers to supply seed to farmers. Many plant pathogens are seed-borne, which can cause enormous crop losses. Out of 16%, annual crop losses due to plant diseases, at least 10% loss is incurred due to seed-borne diseases [1]. Coincidentally important or devastating crop diseases are seed-borne and caused by fungi. It has also been demonstrated that seed-borne fungi are responsible for the poor health of seeds in many crops [2].

Flowers are important for their aesthetic and economic value. Flowers are extensively cultivated in advanced as well as in many developing countries. Floriculture plays a significant role in the economy of these countries. In Bangladesh, the cultivation of flowers is still limited. Although flowers are grown in the home gardens, community centers, schools, and office premises for beautification to a considerable extent in the city areas, its cultivation is not popular yet in rural areas. However, nowadays, commercial cultivation of flowers has increased significantly in the country. Since its inception of more than a decade, commercial floriculture in Bangladesh is about to ensure its contribution to the national economy. Now, the flower is a symbol of not only purity and beauty but also a profitable business. Many unemployed youths can enter the job market through commercial flower cultivation as the flower has a huge demand both at home and abroad. 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 [3]. According to an article in Team India, Flower farming is nearly 5% more profitable than rice cultivation, and 2% more profitable than growing vegetables. Due to the enhanced profitability of flower production over existing crop production, farmers are showing interest in the flower industry. By exporting flowers, our country can also earn a good amount of foreign exchange. Thus, there is a great prospect for flower cultivation in Bangladesh. The common flowers grown in Bangladesh are Rose (*Rosa chinensis*), Tube rose (*Polianthes tuberosa*), Zinnia (*Zinnia elegans*), Calendula (*Calendula officinalis*), Marigold (*Tagetes* spp.), Cosmos (*Cosmos bipinnatus*), Balsam (*Impatiens balsamina*), Sunflower (*Helianthus annuus*), Dahlia (*Dahlia* sp.), Chrysanthemum (*Chrysanthemum sinense*), etc. Among the various flower plants, propagated by true local seed are Calendula, Marigold, Cosmos, Periwinkle and hybrid seed are African marigold, Celosia, Petunia, and *Portulaca* commonly cultivated in the country.

Flowers plants suffer from different diseases like other crop plants. More than 100 diseases in flower plants have been listed by the U.S. Department of Agriculture [4]. Of these, 12 diseases were caused by 20 fungal pathogens, recorded on the eight selected flower plants, Cosmos, Periwinkle, Marigold and calendula are known to be local seed-borne [5]. Sultana [6] recorded five seed-borne fungi on calendula seeds and six seed-borne fungi on cosmos seeds. The prevalence of all fungi recorded varied significantly with respect to flower species and seed

sources. They also found seed germination varied significantly depending on the seed sources and flower species. All these seed-borne pathogens are responsible for causing diseases in the respective flower plants mentioned and incur losses to flower production. Little work has been done on diseases of flower plants in Bangladesh. As such no literature on seed-borne diseases or seed-borne pathogens of flower plants in the country is available. Talukder [7] recorded only a few diseases on some flower plants. Consequently, there is scanty published information on seed-borne diseases or seed health of flower plants propagated by true seeds in Bangladesh. In view of the above facts, the present study has been undertaken to study germination and the prevalence of fungi in seed of some selected local and hybrid flower plants.

2. Materials and Methods

2.1. Collection of Seed Samples

Seeds of eight selected flower plant species were collected from Dhaka districts. Two types of seeds were included in this study such as 1) Hybrid seeds and 2) Local seeds (Table 1). Local seeds were collected from 3 different sources viz. Arboriculture Garden, Ramna Park Garden, Horticulture Garden and hybrid seeds were collected from one source, Momin Beej Ghar, Siddique bazaar. For local seeds, four (4) seed sample were obtained from each of the three (3) seed source. Thus, 12 seed samples obtained for local seeds. For seed samples from hybrid seeds, each sample representing one seed source. Thus, altogether 16 seed samples were collected from eight selected flower plants representing 4 different seed sources; for the present study. After collection, the seeds were kept in polythene bags and brought directly to the Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka. Seeds are stored in plastic or glass container, for both local and hybrid seeds.

Table 1. Local and hybrid flower plants species included for the study.

Local Name	English Name	Scientific Name	Family
Local flowers			
Calendula	Calendula	<i>Calendula officinalis</i>	Compositae
Cosmos	Cosmos	<i>Cosmos bipinnatus</i>	Compositae
Ganda	Marigold	<i>Tagetes erecta</i>	Compositae
Periwinkle	Periwinkle	<i>Vinca rosea</i>	Apocynaceae
Hybrid flowers			
Gada	African Marigold	<i>Tagetes erecta</i>	Compositae
Celosia	Cockscomb	<i>Celosia plumosa</i>	Amaranthaceae
Petunia	Petunia	<i>Petunia ibrida</i>	Miscuglio
Timeful	<i>Portulaca</i>	<i>Portulaca grandiflora</i>	<i>Portulacaceae</i>

2.2. Inspection of Dry Seeds

In this method, only local seeds were taken for inspection. Inspection of dry local seeds was done according to the International Rules of ISTA [8]. In this method, 100 g seeds were taken from local seeds and usually inspected and graded into three categories; 1) Pure seeds 2) Seeds of other flowers 3) Inert matter. The seeds under each category were weighted and calculated the percentages of each group. After recording the data of dry inspection, rest of seeds were kept for blotter test.

2.3. Detection of Seed-Borne Fungi (Blotter Method)

Health of all the seed samples used were analyzed for detection of fungi by the Blotter Method following the International Rules for Seed Testing Association [8]. Three pieces of whatman no. 1 filter paper were soaked in sterilized water and placed at the bottom of a 9 cm plastic Petridish. The seeds were planted on the wet filter paper in the Petridish. Two hundred seeds from each sample were taken randomly and placed on the moist filter paper in 8 replicate petridishes at the rate of 25 seeds per plate. The Petridish with seeds were then incubated at $22^{\circ}\text{C} \pm 2^{\circ}\text{C}$ under 12\12 hours alternating cycle Near Ultra-Violet (NUV) light and dark in the incubation room of the Seed Health Laboratory, SAU, Dhaka for seven days. After incubation, the plates were examined under stereo-microscope for detection of pathogens. Germination % of the seeds was also recorded. The fungi were identified up to the species following the keys of Mathur and Kongsdal [9].

2.4. Agar Plate Technique

In the agar plate method, generally surface-disinfected (0.05% HgCl_2 for 30 sec) seeds were planted on an agar medium and the plated seeds were usually incubated for 5 days at $22^{\circ}\text{C} - 25^{\circ}\text{C}$ under 12 h alternating cycles of light and darkness. At the end of incubation period, fungi growing out from seeds on the agar medium were examined and identified. In this method, Potato Dextrose Agar (PDA) (Potato 200 g, dextrose 20 g, Agar 15 g and H_2O -1000 ml) having pH 6.5 was used. About 15 ml of the media were poured in each sterilized glass Petridish.

2.5. Statistical Analysis

The data were analysis following the Completely Randomized Design (CRD). The mean differences for efficiency of the treatments were judged by least significant difference (LSD) test.

3. Results

The results obtained from present study for dry inspection, different germination rate, seed-borne infection and other analyses have been presented and discussed in this chapter.

3.1. Dry Inspection of Local Seed

The results of dry inspection of seeds of four local flowers collected from three different locations (Arboriculture Garden, Ramna Park Garden and Horticultural Garden) of Dhaka district were presented in **Table 2**. It was observed that three categories of inspections viz. 1) pure seed, 2) inert matter and 3) seeds of other crops were done in respect of individual flower according to the location (**Figure 1**). The percent of pure seeds, inert matter and seeds of other crops respectively were measured from all the samples ranged between 90% - 98.5%, 1.5% - 5.5% and 1% - 3.5% respectively. The highest pure seeds were achieved from Ramna Park Garden for Cosmos (95.5%), Calendula (93%) and Periwinkle (96%) but from Horticultural Garden for Marigold (98.5%). In case of inert matter, the lowest percentage was obtained from Horticultural Garden for Marigold (1.5%) where the highest (5.5%) was also in Calendula from Horticultural Garden. The lowest contamination (1%) of other seeds with the targeted seeds was in Marigold collected from Horticultural Garden but the highest was observed in Calendula collected from Arboriculture Garden, Asadgate.

Table 2. Dry inspection of selected local seed samples of three locations.

Name of the flowerplants	Name of the location	Pure seed (%)	Inert matter (%)	Seeds of other crops (%)
Cosmos	Arboriculture garden	94.50	3.00	2.50
	Ramna park garden	95.50	3.00	1.50
	Horticulture garden	93.00	3.50	3.50
Marigold	Arboriculture garden	97.00	2.00	1.00
	Ramna park garden	96.50	2.50	1.00
	Horticulture garden	97.50	1.50	1.00
Calendula	Arboriculture garden	92.00	4.50	3.50
	Ramna park garden	93.00	3.50	3.50
	Horticulture garden	91.00	5.50	3.50
Periwinkle	Arboriculture garden	95.50	2.50	2.00
	Ramna park garden	96.00	2.00	2.00
	Horticulture garden	95.00	2.50	2.50



Figure 1. Dry inspection of (a) marigold seeds and (b) cosmos seeds.

3.2. Germination of Local Flower and Hybrid Seeds

Blotter Method

1) Cosmos

Percent (%) seed germination of cosmos flower seeds collected from different location such as Arboriculture, Ramna Park and Horticultural Garden in Dhaka district varied significantly at different days after sowing (7 and 14 DAS) (**Table 3**). The germination percentage of this flower seeds collected from three locations was ranged between 88.60% - 91.50% at 7 DAS and 88.25% - 91.38% at 14 DAS. It was observed that the highest germination percentage; 91.50% and 91.38% respectively was in Horticultural garden's seeds at 7 DAS and 14 DAS respectively. On the other hand, the lowest germination percentage; 88.60% and 88.25% respectively was in Arboriculture garden's seeds at 7 DAS and 14 DAS respectively. The results obtained from Ramna park garden's seeds showed intermediate result compared to the germination percentage of Arboriculture and Horticultural garden's seeds.

2) Marigold

Percent (%) seed germination of marigold flower seeds collected from different location such as Arboriculture Garden, Ramna Park and Horticultural Garden in Dhaka district varied significantly at 14 DAS but there was no significant difference at 7 DAS (**Table 3**). The germination percentage of this flower seeds collected from three locations was ranged between 84.15% - 95.25% at 14 DAS.

Table 3. Germination of local and hybrid flower seeds collected from three different locations (blotter method).

Treatments/Locations		% Seed germination	
		7 DAS	14 DAS
Cosmos	Arboriculture garden	88.25 c	88.60 c
	Ramna park	90.00 b	90.00 b
	Horticultural garden	91.38 a	91.50 a
Marigold	Arboriculture garden	95.50 a	95.50
	Ramna park	88.88 b	95.38
	Horticultural garden	84.15 c	96.25
Calendula	Arboriculture garden	64.75 a	74.25 b
	Ramna park	65.88 a	75.88 a
	Horticultural garden	57.50 b	63.63 c
Periwinkle	Arboriculture garden	77.75	84.25 b
	Ramna park	79.38	86.50 a
	Horticultural garden	76.50	85.63 a
Momin Beej Ghar	African marigold	96.00 a	98.25 a
	Celosia	95.70 a	96.00 b
	Petunia	91.25 b	91.38 c
	<i>Portulaca</i>	95.13 ab	95.13 b

It was observed that the highest germination percentage (95.25%) was in Arboriculture garden's seeds where the lowest (84.15%) was in Horticultural garden's seeds at 14 DAS respectively. The results obtained from Ramna park garden's seeds showed intermediate result compared to the germination percentage of Arboriculture and Horticultural garden's seeds.

3) Calendula

Percent (%) seed germination of calendula flower seeds collected from different location such as Arboriculture Garden, Ramna Park and Horticultural Garden in Dhaka district varied significantly at different days after sowing (7 and 14 DAS) (Table 3). The germination percentage of this flower seeds collected from three locations was ranged between 63.63% - 75.88% at 7 DAS and 57.5% - 65.88% at 14 DAS. It was observed that at 7 DAS, the highest germination percentage (75.88%) was in Ramna park garden's seeds where the lowest (63.63%) was in Horticultural garden's seeds. Again, at 14 DAS the highest germination percentage (65.88%) was in Ramna park garden's seeds which were statistically identical with Arboriculture garden's seeds but the lowest was obtained from Horticultural garden's seeds.

4) Periwinkle

Percent (%) seed germination of Periwinkle flower seeds collected from different location such as Arboriculture Garden, Ramna Park and Horticultural Garden in Dhaka district varied significantly at different days after sowing (7 and 14 DAS) (Table 3). The germination percentage of this flower seeds collected from three locations was ranged between 84.25% - 86.5% at 7 DAS and 76.5% - 79.38% at 14 DAS. It was observed that the highest germination percentage at 7 DAS (86.5%) was in Ramna park garden's seeds which were statistically identical with Horticultural garden's seeds but the lowest was obtained from Arboriculture garden's seeds. It was also evident that at 14 DAS there was no significant effect on percentage of Periwinkle seeds germination collected from Arboriculture Garden, Ramna Park and Horticultural Garden.

5) Hybrid flower

Percent (%) seed germination of four hybrid flower seeds such as African marigold, Celosia, Petunia and *Portulaca* varied significantly at different days after sowing (7 and 14 DAS) (Table 3). These four hybrid flower seeds were collected from Momin Beej Ghar. The germination percentage of these four hybrid flower seeds was ranged between 91.25% - 96% at 7 DAS and 91.38% - 98.25% at 14 DAS. It was observed that the highest germination percentage (96%) was in African marigold and Celosia seeds where the lowest (91.25%) was in Petunia seeds at 7 DAS. On the other hand, at 14 DAS the germination percentage (98.25%) was in African marigold seeds but the lowest (95.13%) was in *Portulaca* seeds.

3.3. Seed-Borne Fungi Recorded on Local and Hybrid Flower Seeds

Blotter Method

1) Cosmos

The most predominant fungus of cosmos was *Alternaria alternata*. The prevalence of *Alternaria alternata* ranged between 6.5% - 24.25% (Table 4). The incidence of individual fungi recorded on cosmos seeds varied significantly with respect to sources of seeds collection. The maximum (24.25%) incidence of *Alternaria alternata* was recorded in seeds collected from Arboriculture Garden, Dhaka; while the minimum (6.5%) was found at Horticultural Garden, Dhaka (Figure 2). The highest occurrence of *Aspergillus flavus* (3.88%) and *Aspergillus niger* (3.75%) were recorded at Horticulture Garden, under Dhaka district and the lowest (0.88%) and (1.75%) were founded at Ramna Park Garden and Arboriculture Garden respectively. The highest count of *Fusarium semitectum* was observed at Ramna Park Garden; while the lowest incidence of the fungus (0.88%) was encountered at Horticultural Garden under Dhaka district. The maximum (2.5%) prevalence of *Penicillium* sp. was recorded at Horticultural Garden; while the minimum (0.25%) was found at Ramna Park Garden. In case of *Rhizopus* sp., there was no significant variations were found.

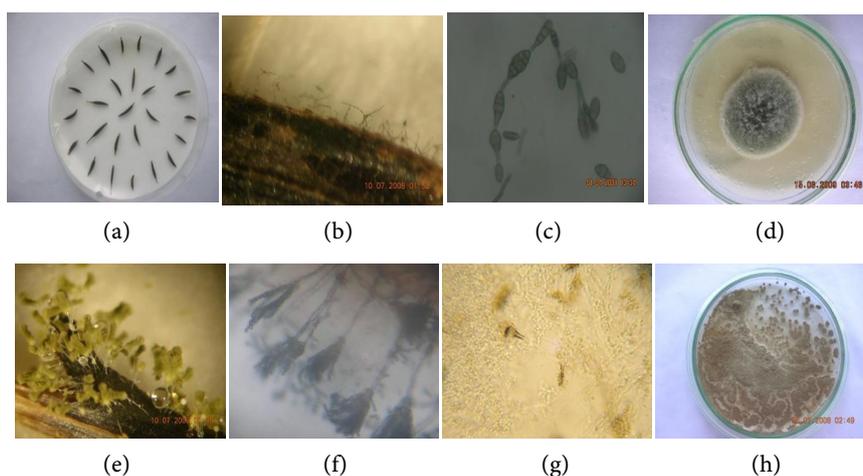


Figure 2. Local seeds were placed on moist blotter paper. (a) Cosmos seeds were placed on blotter paper; (b) Growth of *Alternaria alternata* on a cosmos seed incubated in the blotter (45×); (c) Conidial chain of *Alternaria alternata* (100×); (d) Pure culture of *Alternaria alternata*; (e) Growth of *Penicillium* sp. on a cosmos seed incubated in the blotter (45×); (f & g) Conidia and brush like conidiophore of *Penicillium* sp. (40×); (h) Pure culture of *Penicillium* sp.

Table 4. Prevalence of seed-borne fungi of Cosmos seeds collected from three different sources (blotter method).

Treatments	% Seed-borne infection					
	<i>Alternaria alternata</i>	<i>Penicillium</i> sp.	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus</i> sp.
Arboriculture garden	24.25 a	0.38 b	2.00 b	1.75 b	1.38 b	0.88
Cosmos Ramna park	17.13 b	0.25 b	6.25 a	3.25 ab	0.88 b	0.88
Horticultural garden	6.500 c	2.50 a	0.88 c	3.75 a	3.88 a	0.88
LSD (P = 0.05)	2.594	0.902	0.446	1.801	0.996	NS
CV (%)	2.22	3.43	4.42	2.51	2.44	3.76

2) Marigold

Marigold seeds were tested in blotter method (Figure 3) and the most predominant fungus of marigold within all the pathogens under the experiment was *Alternaria alternata*. Among the pathogens, incidence of *Alternaria alternata* was ranged between 35.88% - 40.97% (Table 5). The incidence of individual fungi recorded on marigold seeds varied significantly with respect to sources of seeds collection. The maximum (40.97% and 2.87% respectively) incidence of *Alternaria alternata* and *Penicillium* sp. respectively was recorded in seeds collected from Arboriculture Garden and Ramna Park Garden respectively under Dhaka which was statistically identical with seeds collected from Horticultural Garden, Dhaka in case of the both pathogens; while the minimum (35.88% and 1.25% respectively) was found at Ramna Park Garden, Arboriculture Garden respectively. The highest occurrence of *Fusarium semitectum* (2.75%), *Aspergillus niger* (2.50%) and *Rhizopus* sp. (1.38%) were recorded from Arboriculture Garden's seeds, Dhaka where the lowest incidence was found in Horticultural Garden's seeds which was statistically identical with seeds collected from Ramna Park, Dhaka. The highest count of *Aspergillus flavus* (7%) was observed in Horticultural Garden's seed; while the lowest was (2%) in Arboriculture garden's seed, Dhaka.

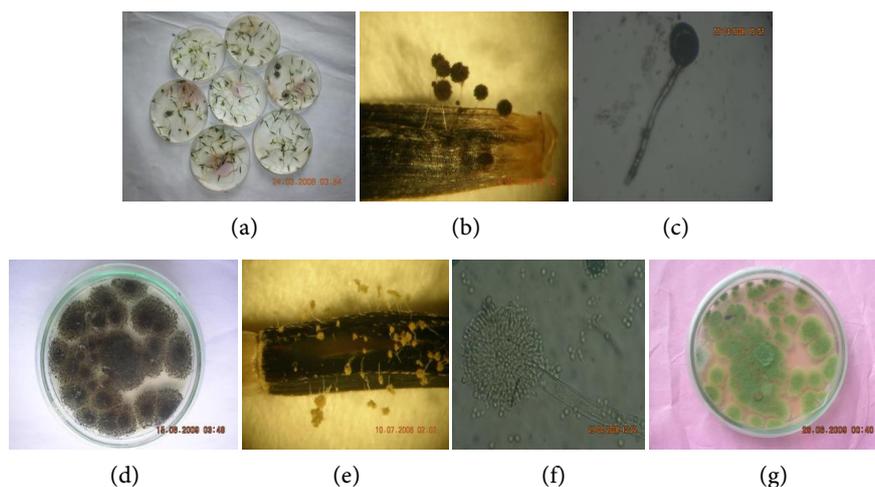


Figure 3. (a) Marigold seeds were germinated on blotter paper; (b) Growth of *Aspergillus niger* on a marigold on blotter (45×); (c) Conidiophore and conidial head of *Aspergillus niger* (40×); (d) Pure culture of *Aspergillus niger*; (e) Growth of *Aspergillus flavus* on a marigold seed on blotter (45×); (f) Conidia and conidiophore of *Aspergillus flavus* (40×); (g) Pure culture of *Aspergillus flavus*.

Table 5. Prevalence of seed-borne fungi of Marigold seeds collected from three different sources (blotter method).

Treatment	% Seed-borne infection					
	<i>Alternaria alternata</i>	<i>Penicillium</i> sp.	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus</i> sp.
Arboriculture garden	40.97 a	1.25 b	2.75 a	2.50 a	2.00 c	1.38 a
Marigold Ramna park	35.88 b	2.88 a	1.25 b	0.88 b	4.75 b	0.88 b
Horticulturalgarden	40.25 a	2.50 a	1.00 b	0.88 b	7.00 a	0.88 b
LSD (P = 0.05)	2.594	0.902	1.412	1.090	1.746	0.357
CV (%)	2.14	2.53	3.87	3.22	3.35	3.99

3) Calendula

Considerable influence was caused by six pathogens under the experiment. The most predominant fungus of calendula was *Fusarium semitectum* (Figure 4). The occurrence of *Fusarium semitectum* ranged between 10% - 22.75% (Table 6). The incidence of individual fungi recorded on calendula seeds varied significantly with respect to sources of seeds collection. The maximum (22.75%) incidence of *Fusarium semitectum* was recorded in seeds collected from Asad-gate Garden, Dhaka which was statistically identical with seeds collected from Ramna Park, Dhaka; while the minimum (10%) was found at Horticultural Garden's seed, Dhaka. Similar results were scrutinized in Ramna park garden's seeds in case of *Alternaria alternata* (4.5%) and *Aspergillus flavus* (3.38%) and were highest occurrence but the lowest were differed between Horticultural Garden and Arboriculture garden's seeds respectively. The fact obtained from Horticultural garden's seeds in case of *Aspergillus niger* (8.25%) and *Rhizopus* sp. (1.38%) were highest compared to Arboriculture and Ramna park garden's seeds. But the fungal effect of *Penicillium* sp. on marigold seeds collected from different location was not substantial.

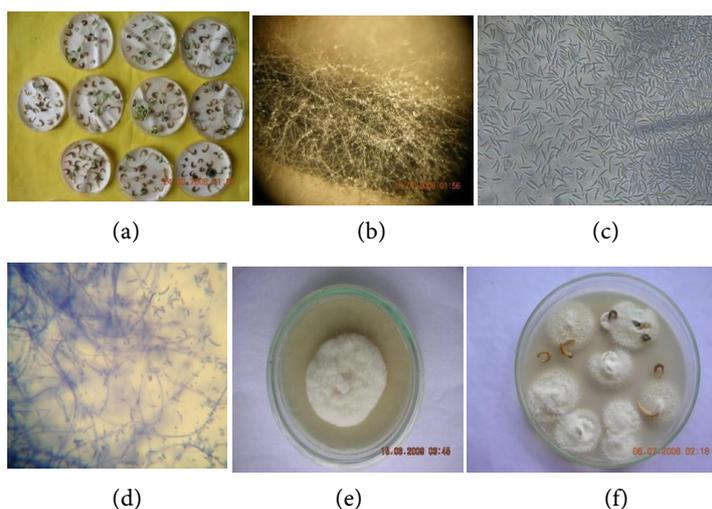


Figure 4. (a) Calendula seeds were germinated on blotter paper; (b) Growth of *Fusarium semitectum* on a calendula seed incubated in the blotter (45×); (c) Conidia of *Fusarium semitectum* (10×); (d) Conidia of *Fusarium semitectum* stained with cotton blue (40×); (e) Pure culture of *Fusarium semitectum*; (f) Calendula seeds were infected by *Fusarium semitectum*.

Table 6. Prevalence of seed-borne fungi of Calendula seeds collected from three different sources (blotter method).

Treatment	% Seed-borne infection					
	<i>Alternaria alternata</i>	<i>Penicillium</i> sp.	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus</i> sp.
Arboriculture garden	3.50 b	0.38	22.75 a	4.38 b	0.38 c	1.00 b
Calendula Ramna park	4.50 a	0.50	21.88 a	5.38 b	3.38 a	0.63 c
Horticultural garden	1.88 c	0.88	10.00 b	8.25 a	2.25 b	1.38 a
LSD (P = 0.05)	0.746	NS	2.471	1.090	0.996	0.357
CV (%)	4.14	3.56	3.71	3.55	2.88	2.18

4) Periwinkle

Fungal effect on periwinkle seeds collected from different location of Dhaka district was literally low compared to all other flower's seeds under the experiment (Table 7). It was evident that there was no significant effect of *Alternaria alternata*, *Penicillium* sp. and *Aspergillus niger* on Periwinkle seeds collected from different location of Dhaka (Arboriculture Garden, Ramna Park and Horticultural Garden). The results obtained from Horticultural garden's seeds in case of *Fusarium semitectum* (2.88%), *Aspergillus flavus* (8.88%) and *Rhizopus* sp. (1.5%) were highest occurrence compared to Arboriculture Garden and Ramna park garden's seeds. But the lowest fungal infestation of *Fusarium semitectum* (0.63%), *Aspergillus flavus* (7%) and *Rhizopus* sp. (0.38%) in periwinkle seeds was found from Arboriculture garden's seed.

5) Hybrid flower

Dominance of seed-borne fungi of some selected hybrid seeds collected from Momin Beej Ghar, Dhaka was tested on blotter (Figure 5) and six fungus infections under the experiment with the determination of pathogenic incidence by blotter method (Table 8). It is evident that the fungal infestation of *Alternaria alternata* and *Aspergillus flavus* was significant among the six pathogens. It was found that the highest incidence of *Alternaria alternata* (1.75%) and *Aspergillus flavus* (1%) was in African marigold seeds where the lowest (0.25% and 0.25% respectively) was in *Portulaca* seeds. The results obtained from African marigold, Celosia, Petunia and *Portulaca* in respect of all the pathogens under the experiment had no significant effect in case of *Penicillium* sp., *Fusarium semitectum*, *Aspergillus niger* and *Rhizopus* sp.

Table 7. Prevalence of seed-borne fungi of Periwinkle seeds collected from three different sources (blotter method).

Treatment	% Seed-borne infection						
	<i>Alternaria alternata</i>	<i>Penicillium</i> sp.	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus</i> sp.	
Arboriculture garden	0.38	0.75	0.63 c	1.13	7.00 c	0.38 c	
Periwinkle	Ramna park	0.75	0.75	1.63 b	1.63	7.75 b	0.75 b
	Horticultural garden	0.50	1.00	2.88 a	1.38	8.88 a	1.50 a
LSD Value (P = 0.05)	NS	NS	0.884	NS	0.796	0.534	
CV (%)	2.14	3.65	3.87	4.29	3.77	2.66	

Table 8. Prevalence of seed-borne fungi of some selected hybrid seeds collected from Momin Beej Ghar (blotter method).

Treatment	% Seed-borne infection						
	<i>Alternaria alternata</i>	<i>Penicillium</i> sp.	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus</i> sp.	
African marigold	1.75 a	0.63	0.25	0.50	1.00 a	0.00	
Momin Beej	Celosia	1.63 a	0.50	0.25	0.38	0.88 a	0.13
	Ghar	Petunia	0.88 b	0.50	0.25	0.25	0.50 b
<i>Portulaca</i>		0.25 c	0.25	0.25	0.25	0.25 c	0.13
LSD Value (P = 0.05)	0.826	NS	NS	NS	0.152	NS	
CV (%)	3.44	4.44	2.72	5.85	4.94	5.31	

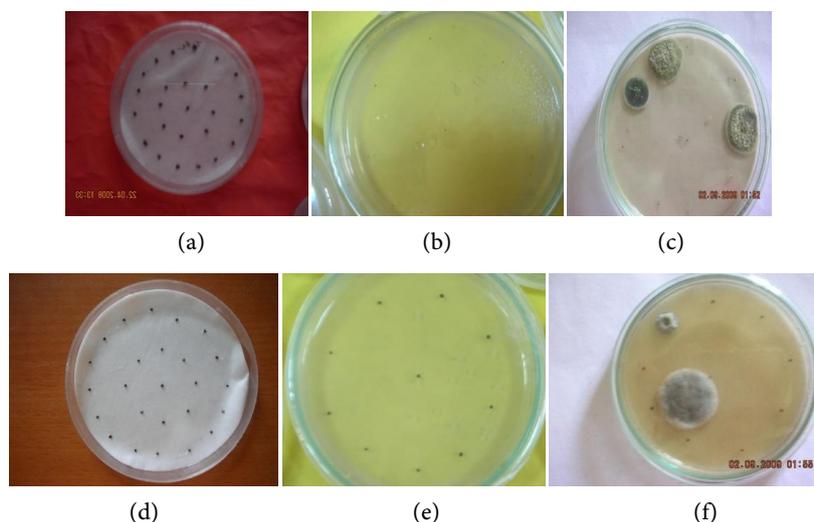


Figure 5. (a) Hybrid seeds were placed on blotter paper (Petunia); (b) Petunia seeds were placed on agar plate; (c) Fungal colonies derived from infected seeds; (d) Hybrid seeds were placed on blotter paper (Celosia); (e) Celosia seeds were placed on agar plate; (f) Fungal colonies derived from infected seeds.

3.4. Agar Plate Method

3.4.1. Cosmos

The most predominant fungus of cosmos was *Alternaria alternata* (Figure 6). The prevalence of *Alternaria alternata* ranged between 15.25% - 16.75% (Table 9). The incidence of individual fungi recorded on cosmos seeds varied significantly with respect to sources of seeds collection. The maximum (16.75%) incidence of *Alternaria alternata* was recorded in seeds collected from Ramna park garden, Dhaka; while the minimum (15.25%) was found at Arboriculture garden's seeds, Dhaka. The highest occurrence of *Penicillium* sp. (4.5%) and *Fusarium semitectum* (2.25%) were recorded at Horticulture Garden, under Dhaka district and the lowest (3.25%) and (0.5%) were found respectively at Arboriculture garden's seed, Dhaka. On the other hand, the highest count of *Aspergillus niger* (7%), *Aspergillus flavus* (9.13%) and *Rhizopus* sp. (2.13%) was observed at Arboriculture garden's seed; while the lowest incidence of the fungus (4.13%), (6.63%) and (0.63%) respectively was encountered at Ramna park garden's seeds under Dhaka district.

3.4.2. Marigold

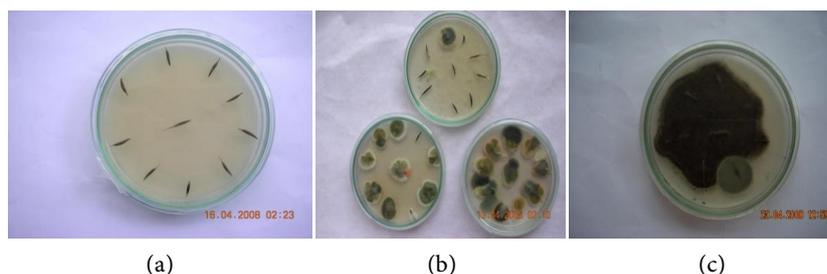
The most predominant fungus of marigold within all the pathogens under the experiment was *Fusarium semitectum*. Among the pathogens, incidence of *Alternaria alternata* was ranged between 18.00% - 21.63% (Table 10). The incidence of individual fungi recorded on marigold seeds varied significantly with respect to sources of seeds collection. The maximum (21.63%) incidence of *Alternaria alternata* was recorded in seeds collected from Ramna park garden's seeds, Dhaka; while the minimum (18%) was found at Arboriculture garden's seeds. Similar result was also found in case of *Fusarium semitectum* where the highest (1.38%) and lowest (0.38%) respectively incidence was recorded at

Table 9. Prevalence of seed-borne fungi of Cosmos seeds collected from three different sources (Agar plate method).

Treatments	% Seed-borne infection					
	<i>Alternaria alternata</i>	<i>Penicillium</i> sp.	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus</i> sp.
Arboriculture garden	15.25 b	3.25 c	0.50 c	7.00 a	9.13 a	2.13 a
Cosmos Ramna park	16.75 a	4.00 b	1.38 b	4.13 c	6.63 b	0.63 b
Horticultural garden	16.00 ab	4.50 a	2.25 a	6.00 b	6.88 b	0.75 b
LSD (P = 0.05)	1.095	0.428	0.276	0.734	1.290	0.464
CV (%)	2.87	3.69	5.12	2.73	4.15	2.21

Table 10. Prevalence of seed-borne fungi of Marigold seeds collected from three different sources (Agar plate method).

Treatment	% Seed-borne infection					
	<i>Alternaria alternata</i>	<i>Penicillium</i> sp.	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus</i> sp.
Arboriculture garden	18.00 c	4.63 a	0.38 c	5.50 a	2.63	0.63
Marigold Ramna park	19.75 b	2.25 b	1.38 a	2.50 c	2.25	0.75
Horticultural garden	21.63 a	2.38 b	0.75 b	4.25 b	2.63	0.63
LSD (P = 0.05)	1.095	0.428	0.276	0.734	NS	NS
CV (%)	4.56	1.11	2.39	3.78	2.45	3.82

**Figure 6.** (a) Cosmos seeds were placed on agar plate; (b) Cosmos seeds were infected by *Aspergillus flavus* and *A. niger*; (c) Fungal colonies derived from infected seeds.

Ramna park garden and Arboriculture garden's seeds respectively. On the other hand, the highest occurrence of *Penicillium* sp. (4.63%) and *Aspergillus niger* (5.5%) were recorded from Arboriculture garden's seeds, Dhaka where the lowest incidence; 2.25% and 2.5% respectively were found in Ramna park garden's seeds, Dhaka. The result obtained from Arboriculture, Ramna Park and Horticultural garden's seeds, Dhaka according to the incidence of *Aspergillus flavus* and *Rhizopus* sp showed statistically insignificant result.

3.4.3. Calendula

Considerable influence was caused by six pathogens under the experiment. The most predominant fungus of calendula was *Fusarium semitectum* and next to *Aspergillus niger*. The occurrence of *Fusarium semitectum* and *Aspergillus niger* respectively were ranged between 9.25% - 11.63% and 6.38% - 8.75% respectively (Table 11). The incidence of individual fungi recorded on calendula seeds varied

significantly with respect to sources of seeds collection. The maximum; 1.25%, 11.63% and 1.75% respectively incidence of *Alternaria alternata*, *Fusarium semitectum* and *Aspergillus flavus* respectively was recorded in seeds collected from Arboriculture Garden, Dhaka and the minimum incidence 0.38%, 9.25% and 0.75% respectively was recorded from Horticultural garden's seeds, Dhaka. On the other hand, the incidence of *Penicillium* sp. (2.88%), *Aspergillus niger* (8.75%) and *Rhizopus* sp. (1.75%) was highest in Ramna park garden's seeds where the lowest (0.25%, 6.38% and 0.12% respectively) was in Arboriculture garden's seeds.

3.4.4. Periwinkle

Fungal effect on periwinkle seeds collected from different location of Dhaka district was significant under the experiment (Table 12). The most predominant fungus of Periwinkle was *Fusarium semitectum* and next to *Aspergillus flavus*. It was evident that the highest effect of *Fusarium semitectum* (6.25%) was in Ramna park garden's seeds which was statistically identical with Horticultural garden's seeds (6%) but the lowest (4.63%) was in Arboriculture garden's seeds. Again, the highest occurrence of *Aspergillus niger* (1.88%) and *Aspergillus flavus* (7%) was in Arboriculture garden's seeds but the lowest (0.38% and 4% respectively) was in Ramna park garden's seeds. It was also observed that there was no significant effect of *Alternaria alternata*, *Penicillium* sp. and *Rhizopus* sp. on periwinkle seeds collected from Arboriculture Garden, Ramna Park and Horticultural Garden, Dhaka.

Table 11. Prevalence of seed-borne fungi of Calendula seeds collected from three different sources (Agar plate method).

Treatment	% Seed-borne infection					
	<i>Alternaria alternata</i>	<i>Penicillium</i> sp.	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus</i> sp.
Arboriculture garden	1.25 a	0.38 b	11.63 a	7.25 b	1.75 a	0.25 b
Calendula Ramna park	0.63 b	2.88 a	10.13 b	8.75 a	1.25 b	1.75 a
Horticultural garden	0.38 b	0.25 b	9.250 c	6.38 c	0.75 c	0.13 b
LSD (P = 0.05)	0.413	0.2759	0.428	0.734	0.473	0.464
CV (%)	3.25	2.24	4.12	3.11	1.26	2.86

Table 12. Prevalence of seed-borne fungi of Periwinkle seeds collected from three different sources (Agar plate method).

Treatment	% Seed-borne infection					
	<i>Alternaria alternata</i>	<i>Penicillium</i> sp.	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus</i> sp.
Arboriculture garden	0.63	0.50	4.63 b	1.88 a	7.00 a	0.25
Periwinkle Ramna park	0.50	0.00	6.25 a	0.38 b	4.00 c	0.00
Horticultural garden	0.25	0.00	6.00 a	1.00 b	4.88 b	0.00
LSD Value (P = 0.05)	NS	NS	0.428	0.734	0.473	NS
CV (%)	3.73	1.34	4.85	4.05	4.61	2.78

3.4.5. Hybrid Flower Seed

Dominance of Seed-borne fungi associated with some selected hybrid seeds collected from Momin Beej Ghar, Dhaka was determined under the experiment by agar plate method (Table 13). It was evident that there was no fungal effect of *Alternaria alternata*, *Fusarium semitectum* and *Rhizopus* sp. in petunia and *Portulaca* seeds, *Penicillium* sp. and *Aspergillus niger* in celosia seeds and *Rhizopus* sp. in African marigold seeds. But the result revealed that the highest infestation of *Alternaria alternata* (3.25%), *Penicillium* sp. (2.63%), *Aspergillus niger* (2.25%) and *Aspergillus flavus* (2.75%) respectively were recorded from African marigold, *Portulaca*, African marigold and Celosia flower's seeds respectively. On the other hand, lowest infestation of *Alternaria alternata* (1.25%), *Penicillium* sp. (1.75%), *Aspergillus niger* (0.63%) and *Aspergillus flavus* (0.75%) respectively were recorded from Celosia, Petunia, *Portulaca* and African marigold flower's seeds respectively. It was also evident that the four hybrid flower seeds collected from Momin Beej Ghar were not significantly influenced by *Fusarium semitectum* and *Rhizopus* sp. among the six pathogens under the experiment.

3.5. Total Infection in Local Flower Seed

Total infection of fungi in different local flower seeds collected from different place in Dhaka district was significant (Table 14). It was observed that incase of pathogenic infection in Cosmos, Marigold, Calendula and Periwinkle seeds the Horticultural garden's seed was better than the others. But according to flower's, Periwinkle was the lowest affected seed and Marigold was the highest affected seed.

3.6. Total Infection in Hybrid Flower Seed

Significant variation was observed in case of total pathogenic infection in hybrid seeds (Table 15). It was observed that the lowest fungal infection (2.82%) was celosia and petunia seeds where the highest (5.82%) was in African marigold seeds.

Table 13. Prevalence of seed-borne fungi of some selected hybrid seeds collected from Momin Beej Ghar (Agar plate method).

Treatment		% Seed-borne infection					
		<i>Alternaria alternata</i>	<i>Penicillium</i> sp.	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus</i> sp.
MominBeej Ghar	African marigold	3.25 a	1.88 b	0.13	2.25 a	0.75 c	0.00
	Celosia	1.25 b	0.00 c	0.13	0.00 d	2.75 a	0.38
	Petunia	0.00 c	1.75 b	0.00	1.38 b	1.38 bc	0.00
	<i>Portulaca</i>	0.00 c	2.63 a	0.00	0.63 c	1.63 b	0.00
LSD Value (P = 0.05)		0.565	0.677	NS	0.611	0.663	NS
CV (%)		3.43	4.30	2.94	5.64	4.29	4.00

Table 14. Prevalence of percent total seed-borne infection in local flower seed collected from different locations.

Treatment	% Seed-borne infection								
	Measured by Blottermethod			Measured by agar platemethod				Average	
	Arboriculture garden	Ramna park garden	Horticultural garden	Arboriculture garden	Ramna park garden	Horticultural garden	Arboriculture garden	Ramna park garden	Horticultural garden
Cosmos	30.64 b	28.64 c	18.39 c	37.26 a	33.52 a	36.38 a	33.95 b	31.08 b	25.33 b
Marigold	48.85 a	41.77 a	45.51 a	31.77 b	28.88 b	32.27 b	40.31 a	35.33 a	31.33 a
Calendula	32.39 b	36.27 b	24.64 b	22.51 c	25.39 c	17.14 c	27.45 c	30.83 b	18.39 c
Nayantara	10.27 c	13.26 d	16.14 c	14.89 d	11.13 d	12.13 d	12.58 d	12.20 d	8.071 d
LSD Value (P = 0.05)	4.632	4.184	3.593	3.892	3.271	3.876	3.388	3.334	4.184
CV (%)	3.362	3.111	2.292	3.674	3.542	4.141	3.582	3.452	4.124

Table 15. Prevalence of percent total seed-borne infection in hybrid flower seed collected.

Treatment	% Seed-borne infection			Average
	Blotter method		Agar plate method	
Momin BeejGhar	African marigold	4.13 a	8.26 a	6.20 a
	Celosia	3.77 b	4.51 b	4.14 b
	Petunia	2.51 c	4.51 b	3.51 c
	<i>Portulaca</i>	1.38 d	4.89 b	3.14 c
LSD Value (P = 0.05)	0.984	1.236	1.142	
CV (%)	3.11	2.59	3.62	

4. Discussions

The present study reveals that there were considerable effects in case of germination percentage and fungal infection occurred on seeds obtaining from dry inspection, agar plate method and blotter method.

The results obtained with dry inspection showed that the purity of different flower seeds collected from different location was acceptable because of percent purity of seeds in every case was in desired value. This was also true incasing of the presence of percent inert matter and presence of other crop seeds.

In the experiment, the germination of seeds of different local flowers collected from different location and different hybrid seeds were measured by only blotter method. Germination percentage of different local seeds and hybrid seeds differed significantly. Results showed that for local flower seeds; Cosmos collected from Horticultural Garden, Marigold collected from a sad gate garden and Calendula and Periwinkle collected from Ramna park garden represented highest germination rate compared to others. This result was occurred due to environmental factors, source of seeds, techniques of seeds growing, post-harvest operation of seed processing etc. But in case of hybrid seeds showed higher percent of

germination because of generally improved techniques are used for hybrid seed processing. The results obtained by Shrotri *et al.* [10] determined the pathogenicity of 20 fungi in calendula seeds and resulted significant reductions in seed-borne fungi and improvements in seed germinability.

The prevalence of the total and the individual fungal infection varied with respect of flower species 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 [11] [12] [13].

Two methods were used for the determination of fungal infection in seeds of different local flower seeds collected from different location and in hybrid seeds. Six fungi; *Alternaria alternata*, *Penicillium* sp., *Fusarium semitectum*, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* sp. were tested for pathogenicity in different seeds of local and hybrid flower seeds.

Result indicated that by Blotter method, the total pathogen for Cosmos, Marigold, Calendula and Nayantar respectively ranged between 18.39% - 30.64%, 41.77% - 48.85%, 24.64 - 36.27% and 10.27% - 16.14% respectively according to different location (Asad gate garden, Ramna park garden and Horticultural Garden of Dhaka district). Again, it was also observed that fungal incidence was higher in Marigold and Periwinkle seeds compared to Cosmos and Calendula due to all three sources of seeds. The results indicated that percent of total seed-borne infection varied with different location but according to local flower seeds, Marigold was infected with highest number of pathogen and Periwinkle was the lowest. Similar phenomenon was not represented by Agar plate method. The total pathogen for Cosmos, Marigold, Calendula and Periwinkle respectively ranged between 33.52% - 37.26%, 28.88% - 32.27%, 17.14 - 25.39% and 11.13% - 14.89% respectively according to different location (Asad gate garden, Ramna park garden and Horticultural Garden of Dhaka district). But according to local flower seeds, Cosmos had the highest pathogen prevalence and Periwinkle had the lowest. It is resulted that Periwinkle showed the lowest pathogen prevalence of four local flower seeds by the both Blotter and Agar plate methods. There are many types of fungal infections occurred by different pathogen in flower seeds [5]. According to Srivastava and Gupta [14] seed-borne fungi *Alternaria alternata*, *Alternaria zinniae*, *Glomerella cingulata*, *Cochlioblous lunata*, *Phoma exigua* and *Fusarium* sp. caused seed rot and death of flower seedlings. The findings of the present study are supported by many scientists [15]-[23].

In case of four hybrid flower seeds (African marigold, Celosia, Petunia and *Portulaca*), the presence of six pathogens (*Alternaria alternata*, *Penicillium* sp., *Fusarium semitectum*, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* sp.) were comparatively very much low. But among the four hybrid flower seeds, African marigold showed the highest incidence of fungi where *Portulaca* showed the lowest. These four hybrid flower seeds were collected from one location but pathogenicity varied due to different types.

5. Conclusion

From the result obtained in the experiment, it can be concluded that in case of pathogenic infection in Cosmos, Marigold, Calendula, and Periwinkle seeds, the Horticultural garden's seed was better than the others. But according to flowers, Periwinkle was the lowest affected seed and Marigold was the highest affected seed. But in the case of hybrid flower seeds, the lowest fungal infection was in *Portulaca* seeds whereas the highest was in African marigold seeds. As the study was limited to one district at four 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, especially the pathogenic ones in flower seeds.

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Authors' Contributions

This work was carried out in collaboration among all authors. Author MK conducted the research work. Author FMA designed and supervised the study and edited the manuscript. Author NA and MHA managed the literature searches. All authors read and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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