

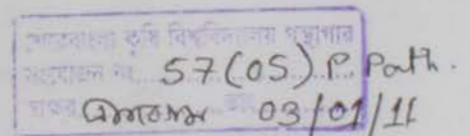
**PREVALENCE OF SEED BORNE FUNGI ASSOCIATED
WITH SEEDS OF SOME SELECTED FLOWERS**

REGISTRATION NO. 07-02614



**DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY**

DHAKA-1207



JUNE, 2009



**PREVALENCE OF SEED BORNE FUNGI ASSOCIATED
WITH SEEDS OF SOME SELECTED FLOWERS**

BY

Registration No. 07-02614

A Thesis

*Submitted to the Faculty of Agriculture,
Sher-e-Bangla Agricultural University, Dhaka
in partial fulfillment of the requirements
for the degree of*

**MASTER OF SCIENCE
IN
PLANT PATHOLOGY**

SEMESTER: JANUARY-JUNE, 2009

Approved by:

Mrs. N. Akhtar

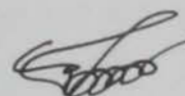
(Mrs. Nasim Akhtar)

Professor

Department of Plant Pathology

Sher-e-Bangla Agricultural University

Supervisor



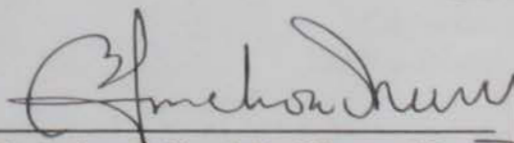
(Dr. F. M. Aminuzzaman)

Assistant Professor

Department of Plant Pathology

Sher-e-Bangla Agricultural University

Co-supervisor



(Dr. M. Salahuddin M. Chowdhury)

Chairman

Examination Committee

Department of Plant Pathology

Sher-e-Bangla Agricultural University



Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

PABX: +88029144270-9
Fax: +88029112649
Web site: www.sau.edu.bd

Ref:

Date: 30-06-2009

CERTIFICATE

This is to certify that the thesis entitled "PREVALENCE OF SEED BORNE FUNGI ASSOCIATED WITH SEEDS OF SOME SELECTED FLOWERS" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of *MASTER OF SCIENCE IN PLANT PATHOLOGY*, embodies the result of a piece of bona fide research work carried out by *Monnuzan Kumkum*, Registration No. 07-02614, under my supervision and guidance. No part of this thesis has been submitted for any other degree or diploma elsewhere.

I further certify that any help or sources of information availed during the course of this inquire have been duly acknowledged and the contents and style of the thesis have been approved and recommended for submission.

Mrs. N. Akhtar

Dated: 30-06-2009

Dhaka, Bangladesh

Professor (Mrs. Nasim Akhtar)
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207
Supervisor

ACKNOWLEDGEMENTS

All praises to Almighty and Kindfull "Allah Rabbul Al-Amin" who enabled me to pursue my higher study and to complete the research work as well as to submit the thesis for the degree of Master of Science (M.S.) in Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh.

The author avails the opportunity of conveying her heartfelt respect, deepest sense of gratitude, immense indebtedness and profound honour to her most reverend teacher and research supervisor, Mrs. Nasim Akhtar, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh for her scholastic guidance, valuable suggestions, constructive criticisms, continuous inspiration and constant encouragement during the entire period of the research work and in the preparation of the manuscript.

The author wishes to express her sincere appreciation and extreme gratitude to her co-supervisor, Dr. F. M. Aminuzzaman, Assistant Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh for his precious advices, instructions, inspirations and cordial help to complete the research work.

Cordial thanks and honors to Dr. M. Salahuddin M. Chowdhury, Chairman, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh for his inspiration and co-operation throughout the period of the research work.

The author expresses heartfelt thanks and gratitude to her esteemed teachers, Dr. Md. Rafiqul Islam, Professor, Nazneen Sultana, Associate Professor and Khadija Akhter, Assistant Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh for their inspiration and co-operation throughout the period of the research work.

The author expresses her sincere appreciation to Abu Noman Faruk Ahammed, Assistant Professor, Md. Belal Hossain, Assistant Professor, Fatema Begum, Assistant Professor, Nazmunnahar Tonu, Assistant Professor and Md. Tohidul

Islam, Assistant Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh for their inspiration and co-operation throughout the period of the research work.

Thanks are extended to Momin Uddin, Pratilata, Rehena Akhter and Khorshed Alam, Laboratory staff, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh for their help and co-operation during the research work.

The author is really grateful to her senior brothers and sisters, Md. Enamul Islam, Alam, Muhibur, Ariful Haque, Nibir shaha, Asad and Boni, Momo, Kamrun, Ferdousi for their co-operation to complete the research work.

The author is also grateful to her friends Hasan, Mishu, Masum, Anju, Raton, Shimu, Beuty, Nasim Haider, Ahsan Habib Rubel, Tanjina, Mili, Minhaz, Dilruba, Sukti, Romana and Jebin for their friendly co-operation.

Specially thanks are extended to her junior brothers and sister Shimul, Arif, Miti and Farhana for their help in compiling and analysis the data.

The author expresses her deepest indebtedness and heartfelt sense of gratitude and special thanks for her father-in-law, mother-in-law, husband Shazzad Hossain for their blessing inspiration and best wishes throughout the study and the author specially grateful her Son Sabhan for his moral support and greatest sacrifices to complete the higher studies successfully.

The Author

CONTENTS

CHAPTER	TITLE	PAGE NO.
	ACKNOWLEDGEMENT	i – ii
	CONTENTS	iii – iv
	LIST OF TABLE	v – vi
	LIST OF PLATES	vii – viii
	LIST OF FIGURES	ix
	ABSTRACT	x
1	INTRODUCTION	1 – 3
2	REVIEW OF LITERATURE	4 – 9
3	MATERIALS AND METHODS	10 – 13
	3.1 Experimental site	10
	3.2 Experimental period	10
	3.3 Collection of seed samples	10
	3.4 Inspection of dry seeds	12
	3.5 Detection of seed borne fungi	12
	3.5.1 Seed health study (Blotter method)	12
	3.6 Agar plate technique	13
	3.7 Statistical analysis	13
4	RESULTS	14 – 49
	4.1 Dry inspection of local seeds	14
	4.2 Germination of local flower and hybrid seeds	18
	4.2.1 Blotter method	18
	4.2.1.1 Cosmos	18
	4.2.1.2 Marigold	19

CHAPTER	TITLE	PAGE NO.
	4.2.1.3 Calendula	20
	4.2.1.4 Periwinkle	21
	4.2.1.5 Hybrid flower	22
	4.3 Seed-borne fungi recorded on local and hybrid flower seeds	23
	4.3.1 Blotter method	23
	4.3.1.1 Cosmos	23
	4.3.1.2 Marigold	28
	4.3.1.3 Calendula	33
	4.3.1.4 Periwinkle	36
	4.3.1.5 Hybrid flower	37
	4.3.2 Agarplate method	39
	4.3.2.1 Cosmos	39
	4.3.2.2 Marigold	41
	4.3.2.3 Calendula	42
	4.3.2.4 Periwinkle	44
	4.3.2.5 Hybrid flower	45
	4.3.2.6 Total infection in local flower seed	48
	4.3.2.7 Total infection in hybrid flower seed	49
5	DISCUSSION	50 – 53
6	SUMMARY AND CONCLUSION	54 – 56
7	LITERATURE CITED	57 – 59

LIST OF TABLES

TABLE NO.	TITLE	PAGE NO.
1	Local flower plants species included in the study	11
2	Hybrid flower plants species included in the study	11
3	List of seed sources included in the study	11
4	Dry inspection of local seed sample of three locations	15
5	Germination of cosmos seeds collected from three different sources (blotter method)	18
6	Germination of marigold seeds collected from three different sources (blotter method)	19
7	Germination of calendula seeds collected from three different sources (blotter method)	20
8	Germination of periwinkle seeds collected from three different sources (blotter method)	21
9	Germination of different hybrid seeds collected from Momin Beej Ghar (blotter method)	22
10	Prevalence of seed-borne fungi of cosmos seeds collected from three different sources (blotter method)	24
11	Prevalence of seed-borne fungi of marigold seeds collected from three different sources (blotter method)	29
12	Prevalence of seed-borne fungi of calendula seeds collected from three different sources (blotter method)	34
13	Prevalence of seed-borne fungi of periwinkle seeds collected from three different sources (blotter method)	36

LIST OF TABLES (continued)

TABLE NO.	TITLE	PAGE NO.
14	Prevalence of seed-borne fungi of some selected hybrid seeds collected from Momin Beej Ghar (blotter method)	37
15	Prevalence of seed-borne fungi of cosmos seeds collected from three different sources (Agar plate method)	39
16	Prevalence of seed-borne fungi of marigold seeds collected from three different sources (Agar plate method)	41
17	Prevalence of seed-borne fungi of calendula seeds collected from three different sources (Agar plate method)	42
18	Prevalence of seed-borne fungi of periwinkle seeds collected from three different sources (Agar plate method)	44
19	Prevalence of seed-borne fungi of some selected hybrid seeds collected from Momin beej Ghar (Agar plate method)	45
20	Prevalence of percent total seed-borne infection in local flower seed collected from different locations	48
21	Prevalence of percent total seed-borne infection in hybrid flower seed	49

LIST OF PLATES

TABLE NO.	TITLE	PAGE NO.
1	Local seeds were placed on blotter paper (A = Cosmos, B = Periwinkle)	25
2	A. Growth of <i>Alternaria alternata</i> on a cosmos seed incubated in the blotter (45X)	26
	B. Conidial chain of <i>Alternaria alternata</i> (40X)	26
	C. Pure culture of <i>Alternaria alternata</i>	26
3	A. Growth of <i>Penicillium</i> sp on a cosmos seed incubated in the blotter (45X)	27
	B. Conidiophore and conidial chain of <i>Penicillium</i> sp under compound microscope (10X)	27
	C. Pure culture of <i>Penicillium</i> sp	27
4	Seeds were germinated on blotter paper (A = Marigold, B = Calendula)	30
5	A. Growth of <i>Aspergillus niger</i> on a marigold seed incubated in the blotter (45X)	31
	B. Conidiophore and conidial head of <i>Aspergillus niger</i> (40X)	31
	C. Pure culture of <i>Aspergillus niger</i>	31
6	A. Growth of <i>Aspergillus flavus</i> on a marigold seed incubated in the blotter (45X)	32
	B. Conidia and conidiophore of <i>Aspergillus flavus</i> (40X)	32
	C. Pure culture of <i>Aspergillus flavus</i>	32
7	A. Growth of <i>Fusarium semitectum</i> on a calendula seed	35

LIST OF PLATES (continued)

TABLE NO.	TITLE	PAGE NO.
7	A. Growth of <i>Fusarium semitectum</i> on a calendula seed incubated in the blotter (45X)	35
	B. Conidia of <i>Fusarium semitectum</i> (10X)	35
	C. Conidia of <i>Fusarium semitectum</i> stained with cotton blue (40X)	35
	D. Pure culture of <i>Fusarium semitectum</i>	35
8	Hybrid seeds were placed on blotter paper (A = Cockscomb, B = Petunia)	38
9	A. Cosmos seeds were placed on agar plate	40
	B. Fungal colonies derived from infected seeds.	40
10	Calendula seeds were infected by <i>Fusarium semitectum</i>	43
11	A. Petunia seeds were placed on agar plate	46
	B. Fungal colonies derived from infected seeds	46
12	A. Cockscomb seeds were placed on agar plate	47
	B. Fungal colonies derived from infected seeds	47

LIST OF FIGURES

TABLE NO.	TITLE	PAGE NO.
1	A. Dry inspection of marigold seeds	16
	B. Pure seeds of marigold seeds	16
	C. Seeds of other crops	16
	D. Inert matter of marigold seeds	16
2	A. Dry inspection of cosmos seeds	17
	B. Pure seeds of cosmos seeds	17
	C. Seeds of other crops	17
	D. Inert matter of cosmos seeds	17

PREVALENCE OF SEED BORNE FUNGI ASSOCIATED WITH SEEDS OF SOME SELECTED FLOWERS

BY

MONNUZAN KUMKUM

ABSTRACT

The prevalence of fungi associated with seeds of some selected local flowers namely cosmos, marigold, calendula and periwinkle collected from three different location (Arboriculture garden, Ramna park garden and Horticulture garden) of 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 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 incase of hybrid seeds, african marigold showed the highest percent seed germination (98.25%). The Horticulture garden's seed was better than the others considering incidence of fungi in all local flower seed 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 incase of hybrid flower seeds, the lowest fungal infection (3.14%) was recorded in portulaca and petunia seeds where the highest (6.20%) was in african marigold seeds.



Dedicated To



My

Respectable

Parents



Chapter 1

Introduction

CHAPTER 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 as 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% losses are incurred due to seed-borne diseases (Fakir, 1983). 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 poor health of seeds in many crops (Neergaard, 1979).

Flowers are important for its aesthetic and economic value. Flowers are extensively cultivated in advanced as well as in many developing countries. Floriculture plays a significant role to the economy of these countries. In Bangladesh, cultivation of flower 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, now-a-days, commercial cultivation of flowers has increased significantly in the country. From its inception more than a decade, commercial floriculture in Bangladesh is about to ensure its contribution to the national economy. Now, flower is a symbol of

not only purity and beauty but also profitable business. Many unemployed youths can enter the job market through commercial flower cultivation as flower has a huge demand both at home and abroad (Hafiz, 2009). 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. Due to enhanced profitability of flower production over existing crop production, farmers are showing interest in 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* sp), 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, cockscomb, petunia, portulaca commonly cultivated in the country.

Flowers plants suffer from different diseases like other crop plants. More than 100 diseases on flower plants have been listed by U.S. Department of Agriculture (USDA, 1960). Of these, 12 diseases caused by 20 fungal pathogens, recorded on the eight selected flowers plants cosmos, periwinkle, marigold and calendula are known to be local seed-borne (Richardson, 1990).

Sultana (2004) recorded five seed borne fungi on calendula seeds and six seed borne fungi on cosmos seeds. 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, (1974) 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 with the following objective:

- To study germination and the prevalence of fungi in seed of the selected local and hybrid flower plants.



Chapter 2

Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

Considerable amount of research works have been done on the seed health or seed-borne nature of fungal pathogens in various crop plants. But a little work has been done on seed-borne diseases and seed health of flower plants in our country. However considerable amount of research works have been conducted on the subject in different parts of the world. Available literatures relevant to this research programme are reviewed in this chapter.

Pape (1942) reported that the pathogen *Alternaria zinniae* was associated with the zinnia seeds. The pathogen was sometimes responsible for rotting of roots and it also produced reddish brown spots on the petals; leaves and stems of *Zinnia elegans*.

Saksena and Singh (1959) observed that marigold blight (*Tagetes erecta*) caused by *Colletotrichum capsici* might be seed-borne. The pathogen was found to infect petiole, peduncle and branches. Prione (1960) reported that blight of zinnia was caused by *Alternaria zinnia* and the causal fungus might be carried with the seed.

U.S. Department of Agriculture listed 12 different diseases on periwinkle, calendula, cosmos, and marigold and zinnia flower plants in the United States (USDA, 1960). The listed diseases were leaf spot (*Cercospora fukushiana*) on balsam, leaf spot (*Alternaria* sp), cercospora leaf spot (*Cercospora calendula*), gray mold blight (*Botrytis cinerea*), leaf and stem

spot (*Colletotrichum gloeosporioides*) on calendula, leaf spot (*Alternaria* sp), head blight (*Botrytis cinerea*), leaf spot (*Cercospora tageticola*), wilt and stem rot (*Fusarium oxysporum*) and flower spot (*Helminthosporium* sp) on marigold and *Alternaria* blight (*Alternaria zinniae*) on zinnia. These diseases are seed-borne. But there was no mention about their transmission through seeds.

Lim (1969) reported *Fusarium oxysporum* sp *callistephi* race 2 causing a wilt of African marigold (*Tagetes erecta*) in Malaya and Singapore. Badly wilted plants showed black unilateral streaks extending upward through the stem from the soil line and pinkish sporodocial spore masses on the lower part of the stem. The same isolate could also infect Chinese aster (*Callisterohus chinensis*). The fungus was reported to be seed-borne.

Talukder (1974) listed some fungal diseases on flower plants in Bangladesh. The diseases were leaf spot (*Alternaria* sp) and petal spot (*Alternaria* sp) on marigold, cercospora spot (*Cercospora* sp) on zinnia and leaf spot disease (*Cercospora impatientis*) on balsam. But, he did not mention anything about the seed-borne nature of these diseases.

From Portugal, Dias *et al.* (1978) reported 42 species of fungi on safflower, tobacco, zinnia, peach, beet and soybean. Of these fungi, *Alternaria carthami* on safflower, *Alternaria longipes* on tobacco, *Alternaria zinniae* on zinnia, *Fusarium amygdali* of peach, *Ramularia beticola* on beet and *Sclerotinia sclerotiorum* on soybean. These were recognized as seed-borne pathogens.

Neergaard (1979) listed two seed-borne fungal pathogens on zinnia. The listed pathogens were *Alternaria zinniae* and *Rhizoctonia solani*.

Srivastava and Gupta (1983) reported that in pathogenicity trials the seed-borne fungi *Alternaria alternata*, *Alternaria zinniae*, *Glomerella cingulata*, *Cochliobolus lunata*, *Phoma exigua* and *Fusarium* sp caused seed rot and death of seedlings, when zinnia seeds were inoculated. Inoculation of aerial parts showed that *A. zinniae*, *A. alternata*, *G. cingulata* and *Myrothecium verrucaria* were severe foliar pathogens, while *P. exigua* caused mild infection.

Shrotri *et al.* (1985) determined the pathogenicity of 20 fungi isolated from calendula seeds. Of the test fungi, *Alternaria alternata*, *Botrytis cinerea*, *Curvularia pallescens*, *Drechslera (Cochliobolus) hawaiiensis* and *Fusarium* sp were pathogenic causing diseases to calendula. Significant reductions in seed borne fungi and improvements in seed germinability were obtained by treatments with Ceresin dry (Methoxyethyl mercury chloride) and Dithane M-45 (Mancozeb).

Prasad (1987) isolated *Alternaria alternata* and *Cladosporium* sp most frequently from seeds of zinnia followed by *A. tenuissima*. Seeds of zinnia occasionally contained *Fusarium moniliforme*.

Karlatti and Hiremath (1989) isolated *Alternaria zinniae* from seeds of marigold plants (*Tagetes erecta*) heavily infected by *Alternaria zinniae* from a garden in Dharwad, Karnatak, India. Seeds collected from the infected plants were separated, dried and plated on potato dextrose agar (PDA). Some of the seeds were surface sterilized. Spore suspensions were prepared and inoculated into seedlings of 10 plants belonging to the Asteraceae.

Alternaria zinniae was successfully isolated from apparently healthy and discoloured seeds and from those that had been surface sterilized. The isolated fungus infected ageratum, aster, chrysanthemum, cosmos and sunflower seedlings.

Richardson (1990) listed 20 major fungal pathogens on the five selected flower plants included in the present study. *Alternaria zinniae* and *Fusarium* sp, were recorded on calendula seed, *Alternaria zinniae*, *Botrytis cinerea* on cosmos seed, *Alternaria zinniae*, *Phyllosticta impatientis* and *Rhizoctonia solani* on balsam seed, *Alternaria tagtica*, *Alternaria zinniae*, *Fusarium oxysporum*, *Rhizoctonia solani*, *Septoria tageticola* on marigold seed and *Alternaria zinniae*, *Botrytis cinerea*, *Colletotrichum acupatum*, *Glomerella cingulata*, *Phyllosticta* sp and *Rhizoctonia solani* on zinnia seed.

In Taiwan Chou and Wu (1995) detected twenty four fungi from 22 seed samples belonging to 13 species of flower crops imported from different countries. Among the isolated fungi *Collectotrichum dematium* from seeds of celosia and globe amaranth, *Alternaria carthami* was from zinnia seed, *Collectotrichum dematium* from seeds of celosia and globe amaranth, *Curvaularia lunata* (*Cochliobolus lunata*) from the seeds of *Tagetes patula* and *Gomphorena globosa*, *Drechslera rostrata* (*Setosphaeria rostrata*) from *Tagetes patula* seed and *Phoma* sp from the seeds of *G. globosa*. These fungi significantly ($P = 0.05$) decreased the rate of germination. In addition, *A. carthami* and *C. dematium* caused disease in zinnia and celosia, respectively. It was claimed that these fungi were reported for the first time as seed borne fungal pathogens respective of the flower crop species internationally. Several other unreported seed-borne fungi were present in flower seeds examined, but they were shown to be non-pathogenic.

Wu *et al.* (1996) were recorded on 15 different species in 13 genera of crops grown for their flowers were health-tested by a blotter method. Thirty-one different species of fungi in 19 genera were found in these seeds. Among them, *Alternaria cosmosa* on yellow cosmos and *Colletotrichum dematium* on pansy were new records of seed-borne pathogens. Newly recorded seed-borne fungi existed in seeds of the all species of flowering species tested except for ageratum, baby's breath, salvia, statice and stokesia.

Chese (1998) observed that the flowing plants, marigold, zinnia, impatiens, pot marigold known to be attacked by *Alternaria* sp were also found to be attacked by *Alternaria tagetes*, *Alternaria zinniae* and *Alternaria* sp. All the *Alternaria* species were seed-borne.

Keisuke *et al.* (1998) observed on African marigold (*Tagetes erecta*) and French marigold (*T. patula*) grown in Miyagi Prefecture, Japan. Similar lesions also developed on stems and flowers, resulting in early blight of the affected organs. Plants with numerous lesions withered rapidly. A mitosporic fungus isolated repeatedly from the diseased plants was identified as *Alternaria tagetica* and demonstrated to cause the disease. The disease, as well as the fungus, is new to Japan.

Wu *et al.* (2001) isolated *Nimbya gomphrenae*, *Stemphylium vesicarium* and *Alternaria tagetica* for the first time from seeds of diseased globe amaranth (*Gomphorena globosa*), pot marigold (*Calendula officinalis*) and marigold (*Tagetes erecta*), respectively in Taiwan. The amount of seed-borne *Alternaria alternata* or *S. vesicarium* inocula was negatively correlated with emergence rate of pot marigold.

Javaid *et al.* (2008) found that *Fusarium oxysporum* f. sp *gladioli* (Massey) Snyder. & Hans. from diseased corms of gladiolus (*Gladiolus grandiflorus* sect. *Blandus*) cv. Aarti.



Chapter 3

Materials and Methods

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental site

The laboratory experiments on the detection of fungi on flower seeds were conducted in Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.

3.2 Experimental period

The experiment was conducted during the period from January, 2008 to March, 2009.

3.3 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 (i) Hybrid seeds and (ii) Local seeds (Table 1 and Table 2). Local seeds were collected from 3 different sources (Table 3) and hybrid seeds were collected from one source; 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 sources. 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 & brought directly to the laboratory of the Seed Health Laboratory SAU, Dhaka. Seeds are stored in plastic or glass container, for both local & hybrid seeds.

Table 1. Local flower plants species included in the study

Local Name	English Name	Scientific Name	Family
Calendula	Calendula	<i>Calendula officinalis</i>	Compositae
Cosmos	Cosmos	<i>Cosmos bipinnatus</i>	Compositae
Gada	Marigold	<i>Tagetes erecta</i>	Compositae
Nayantara	Periwinkle	<i>Vinca rosea</i>	Apocynaceae

Table 2. Hybrid flower plants species included in the study

Local Name	English Name	Scientific Name	Family
Gada	African Marigold	<i>Tagetes erecta</i>	Compositae
Celosia	Cockscomb	<i>Celosia plumosa</i>	Amaranthaceae
Petunia	Petunia	<i>Petunia ibrida</i>	Miscuglio
Timeful	Portulaca	<i>Portulaca grandiflora</i>	Portulacaceae

Table 3. List of seed sources included in the study

SL no.	Seed	District	Source
1	Local seed	Dhaka	(i) Horticulture Garden, Asadgate.
			(ii) Arboriculture Garden, Asadgate.
			(iii) Ramma Park Garden, Ramma Park.
2	Hybrid seed	Dhaka	(i) Momin Beej Ghar, Siddique Bazar.

3.4 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 (1999). In this method, 100 gm seeds were taken from local seeds and usually inspected and graded into three categories; (i) Pure seeds (ii) Seeds of other flowers (iii) 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.

3.5 Detection of Seed-borne fungi

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 (ISTA, 2005).

3.5.1 Seed health study (Blotter method)

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 plated 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 $22 \pm 2^{\circ}\text{c}$ 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 (2003).

3.6 Agar plate technique

In the agar plate method generally surface-disinfected (0.05% Hg₂Cl₂ for 30 sec) seeds were plated on an agar medium and the plated seeds were usually incubated for 5-7 days at 22-25°C under 12h 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 gm, Dextrose 20 gm, Agar 15 gm and H₂O-1000 ml) having pH 6.5 was used. About 15ml of the media were poured in each sterilized glass petridish.

3.7 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.



Chapter 4

Results

CHAPTER 4

RESULTS

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

4.1 Dry inspection of local seed

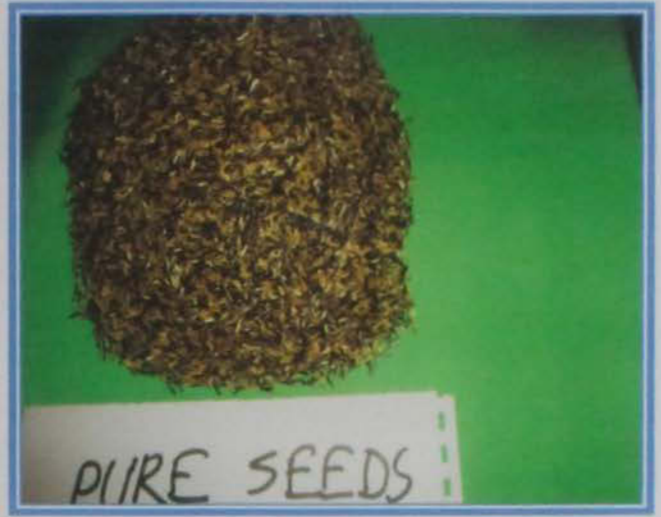
The results of dry inspection of seeds of four local flowers collected from three different locations (Arboriculture Garden, Ramna park garden and Horticulture garden) of Dhaka district were presented in Table 4. It was observed that three categories of inspections viz. (i) pure seed, (ii) inert matter and (iii) seeds of other crops were done in respect of individual flower according to the location (Fig.1 and Fig.2). The percent of pure seeds, inert matter and seeds of other crops were measured from all the samples ranged between 91.00% - 97.00%, 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 Horticulture garden for marigold (97.5%). In case of inert matter, the lowest percentage was obtained from Horticulture garden for marigold (1.5%) where the highest (5.5%) was also in calendula from Horticulture garden. The lowest contamination (1%) of other seeds with the targeted seeds was in marigold collected from Horticulture garden but the highest was observed in calendula collected from Arboriculture Garden, Asadgate.

Table 4. Dry inspection of selected local seed samples of three locations

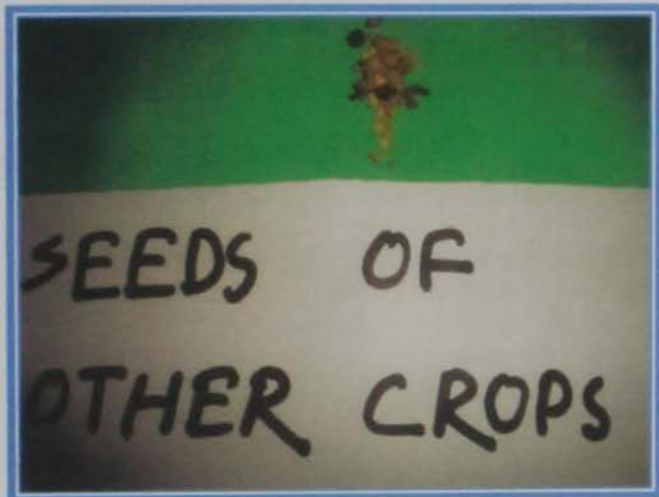
Sl. No.	Name of the flower plants	Name of the location	Pure seed (%)	Inert matter (%)	Seeds of other crops (%)
1.	Cosmos	Arboriculture garden	94.50	3.00	2.50
		Ramna park garden	95.50	3.00	1.50
		Horticulture garden	93.00	3.50	3.50
2.	Marigold	Arboriculture garden	97.00	2.00	1.00
		Ramna park garden	96.50	2.50	1.00
		Horticulture garden	97.50	1.50	1.00
3.	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
4.	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



A



B



C



D

Fig.1. A. Dry inspection of marigold seeds
B. Pure seeds of marigold seeds
C. Seeds of other crops
D. Inert matter of marigold seeds



A



B



C



D

Fig. 2. A. Dry inspection of cosmos seeds
B. Pure seeds of cosmos seeds
C. Seeds of other crops
D. Inert matter of cosmos seeds

4.2 Germination of local flower and hybrid seeds

4.2.1 Blotter method

4.2.1.1 Cosmos

Percent (%) seed germination of cosmos flower seeds collected from different location such as Arboriculture, Ramna park and Horticulture garden in Dhaka district varied significantly at different days after sowing (7 and 14 DAS) (Table 5). 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% was recorded in Horticulture garden's seeds at 7 DAS and 14 DAS respectively. On the other hand the lowest germination percentage; 88.60% and 88.25% was recorded 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 Horticulture garden's seeds.

Table 5. Germination of cosmos seeds collected from three different sources (blotter method)

Treatment (Location)	% Seed germination	
	7 DAS	14 DAS
Arboriculture garden	88.60 c	88.25 c
Ramna park garden	90.00 b	90.00 b
Horticulture garden	91.50 a	91.38 a
LSD Value	1.090	1.333
CV (%)	3.42	4.12

4.2.1.2 Marigold

Percent (%) seed germination of marigold flower seeds collected from different location such as Arboriculture garden, Ramna Park and Horticulture garden in Dhaka district varied significantly at 14 DAS but there was no significant difference at 7 DAS (Table 6). The germination percentage of this flower seeds collected from three locations was ranged between 84.15% - 95.25% at 14 DAS. It was observed that the highest germination percentage (95.25%) was recorded in Arboriculture garden's seeds where the lowest germination (84.15%) was in Horticulture garden's seeds at 14 DAS. The results obtained from Ramna park garden's seeds showed intermediate result compared to the germination percentage of Arboriculture and Horticulture garden's seeds.

Table 6. Germination of marigold seeds collected from three different sources (blotter method)

Treatment (Location)	% Seed germination	
	7 DAS	14 DAS
Arboriculture garden	95.50	95.25 a
Ramna park	95.38	88.88 b
Horticulture garden	96.25	84.15 c
LSD Value	NS	1.426
CV (%)	2.16	3.22

4.2.1.3 Calendula

Percent (%) seed germination of calendula flower seeds collected from different location such as Arboriculture garden, Ramna park and Horticulture garden in Dhaka district varied significantly at different days after sowing (7 and 14 DAS) (Table 7). 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 Horticulture 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 Horticulture garden's seeds.

Table 7. Germination of calendula seeds collected from three different sources (blotter method)

Treatment (Location)	% Seed germination	
	7 DAS	14 DAS
Arboriculture garden	74.25 b	64.75 a
Ramna park garden	75.88 a	65.88 a
Horticulture garden	63.63 c	57.50 b
LSD Value	1.084	1.336
CV (%)	3.51	2.63

4.2.1.4 Periwinkle

Percent (%) seed germination of periwinkle flower seeds collected from different location such as Arboriculture garden, Ramna park and Horticulture garden in Dhaka district varied significantly at different days after sowing (7 and 14 DAS) (Table 8). 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 Horticulture 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 Horticulture garden.

Table 8. Germination of periwinkle seeds collected from three different sources (blotter method)

Treatment (Location)	% Seed germination	
	7 DAS	14 DAS
Arboriculture garden	84.25 b	77.75
Ramna park garden	86.50 a	79.38
Horticulture garden	85.63 a	76.50
LSD Value	1.074	NS
CV (%)	3.33	3.84

4.2.1.5 Hybrid flower

Percent (%) seed germination of four hybrid flower seeds such as african marigold, cockscomb, petunia and portulaca varied significantly at different days after sowing (7 and 14 DAS) (Table 9). 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 cockscomb 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.

Table 9. Germination of different hybrid seeds collected from Momin Beej Ghar (blotter method)

Treatment (Location)	% Seed germination	
	7 DAS	14 DAS
African marigold	96.00 a	98.25 a
Cockscomb	96.00 a	95.75 b
Petunia	91.25 b	91.38 c
Portulaca	95.13 ab	95.13 b
LSD Value	3.995	1.554
CV (%)	3.26	2.21

4.3 Seed-borne fungi recorded on local and hybrid flower seeds

4.3.1 Blotter method

4.3.1.1 Cosmos

The most predominant fungus of cosmos was *Alternaria alternata*. The prevalence of *Alternaria alternata* ranged between 6.5% – 24.25% (Table 10). The incidence of individual fungi recorded on cosmos seeds varied significantly with respect to sources of seeds collection (Plate 1). 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 Horticulture Garden, Dhaka (Plate 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 Horticulture Garden under Dhaka district. The maximum (2.5%) prevalence of *Penicillium* sp was recorded at Horticulture Garden; while the minimum (0.25%) was found at Ramna Park Garden (Plate 3). In case of *Rhizopus* sp there was no significant variations was found.

Table 10. Prevalence of seed-borne fungi of cosmos seeds collected from three different sources (blotter method)

Treatment (Location)	% of seed-borne infection					
	<i>Alternaria alternata</i>	<i>Penicillium sp</i>	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus sp</i>
Arboriculture garden	24.25 a	0.38 b	2.00 b	1.75 b	1.38 b	0.88
Ramna park garden	17.13 b	0.25 b	6.25 a	3.25 ab	0.88 b	0.88
Horticulture garden	6.50 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

57(05) 03/01/11

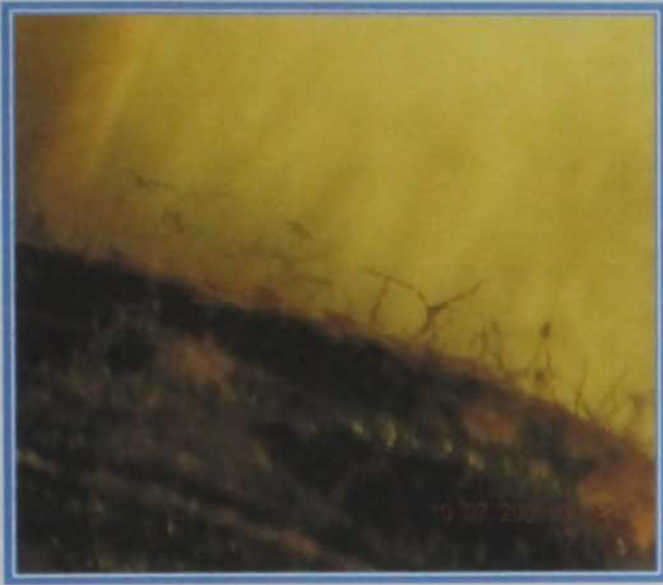


A



B

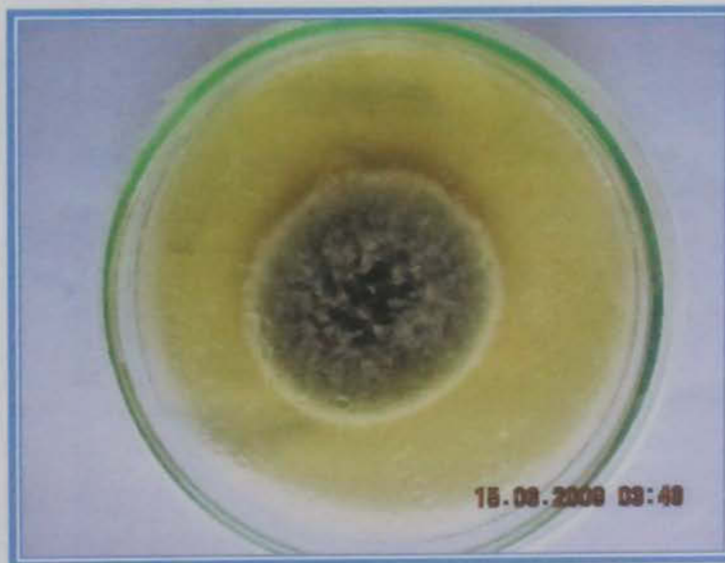
Plate.1. Local seeds were placed on blotter paper
(A=Cosmos, B=Periwinkle)



A



B



C

Plate. 2. A. Growth of *Alternaria alternata* on a cosmos seed incubated in the blotter (45X)

B. Conidial chain of *Alternaria alternata* (40X)

C. Pure culture of *Alternaria alternata*



A



B



C

Plate. 3. A. Growth of *Penicillium* sp on a cosmos seed incubated in the blotter (45X)

B. Conidiophore and conidial chain of *Penicillium* sp under compound microscope (40X)

C. Pure culture of *Penicillium* sp

4.3.1.2 Marigold

Marigold seeds were tested in blotter method (Plate 4) 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 11). The incidence of individual fungi recorded on marigold seeds varied significantly with respect to sources of seeds collection. The maximum (40.97% and 2.88%) incidence of *Alternaria alternata* and *Penicillium* sp was recorded in seeds collected from Arboriculture garden and Ramna Park Garden respectively under Dhaka which was statistically identical with seeds collected from Horticulture Garden, Dhaka incase of the both pathogens; while the minimum (35.88% and 1.25%) was found at Ramna Park Garden, Arboriculture garden seeds, respectively. The highest occurrence of *Fusarium semitectum* (2.75%), *Aspergillus niger* (2.50%) (Plate 5) and *Rhizopus* sp (1.38%) were recorded from Asadgate Garden's seeds, Dhaka where the lowest incidence were found in Horticulture Garden's seeds which was statistically identical with seeds collected from Ramna Park, Dhaka. The highest count of *Aspergillus flavus* (7%) was observed in Horticulture Garden's seed; while the lowest was (2%) in Arboriculture garden's seed, Dhaka (Plate 6).

Table 11. Prevalence of seed-borne fungi of marigold seeds collected from three different sources (blotter method)

Treatment (Location)	% of seed-borne infection					
	<i>Alternaria alternata</i>	<i>Penicillium sp</i>	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus sp</i>
Arboriculture garden	40.97 a	1.25 b	2.75 a	2.50 a	2.00 c	1.38 a
Ramna park garden	35.88 b	2.88 a	1.25 b	0.88 b	4.75 b	0.88 b
Horticulture garden	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



A



B

Plate. 4. Seeds were germinated on blotter paper
(A = Marigold, B = Calendula)



A



B

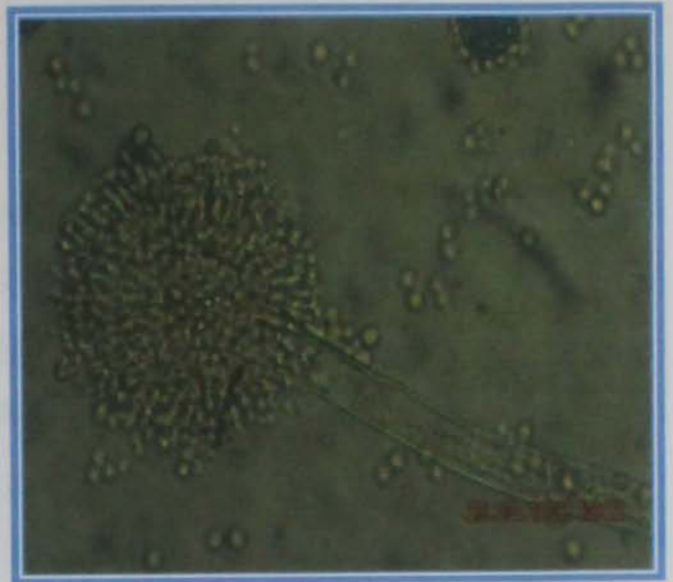


C

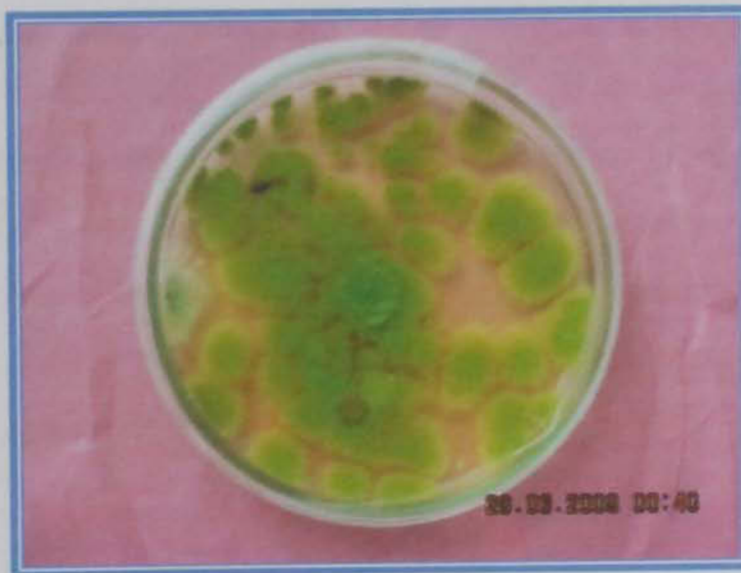
- Plate. 5. A. Growth of *Aspergillus niger* on a marigold seed incubated in the blotter (45X)
B. Conidiophore and conidial head of *Aspergillus niger* (40X)
C. Pure culture of *Aspergillus niger*



A



B



C

Plate. 6. A. Growth of *Aspergillus flavus* on a marigold seed incubated in the blotter (45X)
B. Conidia and conidiophore of *Aspergillus flavus* (40X)
C. Pure culture of *Aspergillus flavus*

4.3.1.3 Calendula

Considerable influence was caused by six pathogens under the experiment. The most predominant fungus of calendula was *Fusarium semitectum* (Plate 7). The occurrence of *Fusarium semitectum* ranged between 10% – 22.75% (Table 12). 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 Arboriculture, Dhaka which was statistically identical with seeds collected from Ramna Park, Dhaka; while the minimum (10%) was found at Horticulture 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 Horticulture garden and Arboriculture garden's seeds respectively. The fact obtained from Horticulture 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.

Table 12. Prevalence of seed-borne fungi of calendula seeds collected from three different sources (blotter method)

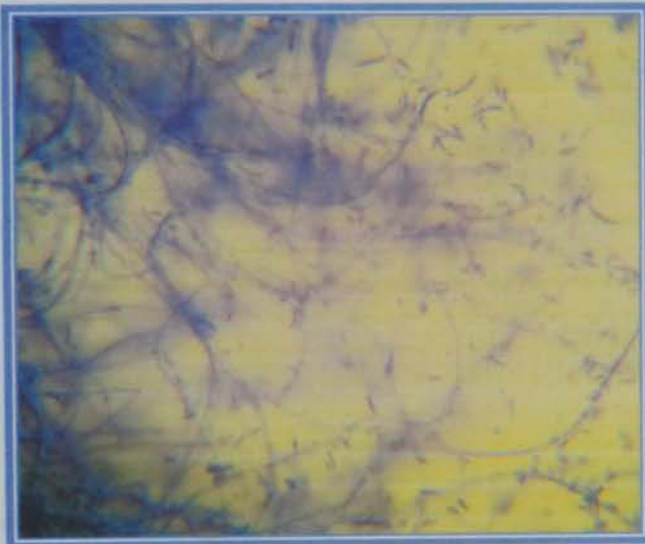
Treatment (Location)	% of seed-borne infection					
	<i>Alternaria alternata</i>	<i>Penicillium</i> sp	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus</i> sp
Arboriculture garden	3.50 b	0.38	22.75 a	4.38 b	0.38 c	1.00 b
Ramna park garden	4.50 a	0.50	21.88 a	5.38 b	3.38 a	0.63 c
Horticulture 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



A



B



C



D

- Plate.7. A. Growth of *Fusarium semitectum* on a calendula
Seed incubated in the blotter (45X)
B. Conidia of *Fusarium semitectum* (10X)
C. Mycelia and conidia of *Fusarium semitectum* stained
with cotton blue (40X)
D. Pure culture of *Fusarium semitectum*

4.3.1.4 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 13). 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 Horticulture 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.

Table 13. Prevalence of seed-borne fungi of periwinkle seeds collected from three different sources (blotter method)

Treatment (Location)	% of seed-borne infection					
	<i>Alternaria alternata</i>	<i>Penicillium sp</i>	<i>Fusarium semitectum</i>	<i>A. niger</i>	<i>A. flavus</i>	<i>Rhizopus sp</i>
Arboriculture garden	0.38	0.75	0.63 c	1.13	7.00 c	0.38 c
Ramna park garden	0.75	0.75	1.63 b	1.63	7.75 b	0.75 b
Horticulture 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 (%)	2.07	3.93	3.69	4.92	2.52	4.01

4.3.1.5 Hybrid flower

Dominance of seed borne fungi of some selected hybrid seeds collected from Momin Beej Ghar, Dhaka was tested on blotter (Plate 8) and six fungus infections under the experiment with the determination of pathogenic incidence by blotter method (Table 14). 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 14. Prevalence of seed-borne fungi of some selected hybrid seeds collected from Momin Beej Ghar (blotter method)

Treatment	% of 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
Cockscomb	1.63 a	0.50	0.25	0.38	0.88 a	0.13
Petunia	0.88 b	0.50	0.25	0.25	0.50 b	0.13
Portulaca	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



A



B

Plate.8. Hybrid seeds were placed on blotter paper
(A = Celosia, B = Petunia)

4.3.2 Agar plate method

4.3.2.1 Cosmos

The most predominant fungus of cosmos was *Alternaria alternata*. The prevalence of *Alternaria alternata* ranged between 15.25% – 16.75% (Table 15). 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 occurrences of *Fusarium semitectum* (2.25%) were recorded at Horticulture garden, under Dhaka district and the lowest (0.5%) were found at Arboriculture garden's seed, Dhaka. On the other hand the highest count of *Aspergillus niger* (7%) was observed in Arboriculture garden's seed; while the lowest incidence of the fungus (4.13%) was encountered at Ramna park garden's seeds under Dhaka district (Plate 9).

Table 15. Prevalence of seed-borne fungi of cosmos seeds collected from three different sources (Agar plate method)

Treatment (Location)	% of seed-borne infection		
	<i>Alternaria alternata</i>	<i>Fusarium semitectum</i>	<i>A. niger</i>
Arboriculture garden	15.25 b	0.50 c	7.00 a
Ramna park garden	16.75 a	1.38 b	4.13 c
Horticulture garden	16.00 ab	2.25 a	6.00 b
LSD (P=0.05)	1.095	0.276	0.734
CV (%)	2.87	5.12	2.73



A



B

Plate. 9. A. Cosmos seeds were placed on agar plate
B. Fungal colonies derived from infected seeds

4.3.2.2 Marigold

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 18.00% – 21.63% (Table 16). The incidence of individual fungi recorded on marigold seeds varied significantly with respect to sources of seed collection. The maximum (21.63%) incidence of *Alternaria alternata* was recorded in seeds collected from Horticulture garden's seeds, Dhaka; while the minimum (18%) was found at Arboriculture garden's seeds. Similar result was also found incase of *Fusarium semitectum* where the highest (1.38%) and the lowest (0.38%) respectively incidence was recorded at Ramna park garden and Arboriculture garden's seeds, respectively.

Table 16. Prevalence of seed-borne fungi of marigold seeds collected from three different sources (Agar plate method)

Treatment (Location)	% of seed-borne infection	
	<i>Alternaria alternata</i>	<i>Fusarium semitectum</i>
Arboriculture garden	18.00 c	0.38 c
Ramna park garden	19.75 b	1.38 a
Horticulture garden	21.63 a	0.75 b
LSD (P=0.05)	1.095	0.276
CV (%)	4.56	2.39

4.3.2.3 Calendula

The most predominant fungus of calendula was *Fusarium semitectum*. The occurrence of *Fusarium semitectum* was ranged between 9.25% – 11.63% (Table 17). The incidence of individual fungi recorded on calendula seeds varied significantly with respect to sources of seeds collection. The maximum 1.25% and 11.63% incidence of *Alternaria alternata* and *Fusarium semitectum* was recorded in seeds collected from Arboriculture garden, Dhaka and the minimum incidence 0.38% and 9.25% was recorded from Horticulture garden's seeds, Dhaka. The growth of *Fusarium semitectum* associated with calendula seeds on agar plate were shown in plate 10.

Table 17. Prevalence of seed-borne fungi of calendula seeds collected from three different sources (Agar plate method)

Treatment (Location)	% of seed-borne infection	
	<i>Alternaria alternata</i>	<i>Fusarium semitectum</i>
Arboriculture garden	1.25 a	11.63 a
Ramna park garden	0.63 b	10.13 b
Horticulture garden	0.38 b	9.25 c
LSD (P=0.05)	0.413	0.428
CV (%)	3.25	4.12



Plate.10. Calendula seeds were infected by *Fusarium semitectum*

4.3.2.4 Periwinkle

Fungal effect on periwinkle seeds collected from different location of Dhaka district was significant under the experiment (Table 18). The most predominant fungus of periwinkle was *Fusarium semitectum*. It was evident that the highest incidence of *Fusarium semitectum* (6.25%) was in Ramna park garden's seeds which was statistically identical with Horticulture garden's seeds (6%) but the lowest (4.63%) was recorded in Arboriculture garden's seeds. Again, the highest occurrence of *Aspergillus niger* (1.88%) was in Arboriculture garden's seeds but the lowest (0.38%) was in Ramna park garden's seeds. It was also observed that there was no significant effect of *Alternaria alternata* on periwinkle seeds collected from Arboriculture garden, Ramna park and Horticulture garden, Dhaka.

Table 18. Prevalence of seed-borne fungi of periwinkle seeds collected from three different sources (Agar plate method)

Treatment (Location)	% of seed-borne infection		
	<i>Alternaria alternata</i>	<i>Fusarium semitectum</i>	<i>A. niger</i>
Arboriculture garden	0.63	4.63 b	1.88 a
Ramna park garden	0.50	6.25 a	0.38 b
Horticulture garden	0.25	6.00 a	1.00 b
LSD Value (P=0.05)	NS	0.428	0.734
CV (%)	3.73	4.85	4.05

4.3.2.5 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 19; Plate 11 and Plate 12). It was evident that there was no fungal effect of *Alternaria alternata* and *Fusarium semitectum* in petunia and portulaca seeds, *Penicillium* sp in cockscomb seeds. But the result revealed that the highest infestation of *Alternaria alternata* (3.25%), *Penicillium* sp (2.63%) and *Aspergillus flavus* (2.75%), were recorded from african marigold, portulaca, and cockscomb flower's seeds, respectively. On the other hand the lowest infestation of *Alternaria alternata* (1.25%), *Penicillium* sp (1.75%) and *Aspergillus flavus* (0.75%) were recorded from cockscomb, petunia 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* among the four pathogens under the experiment.

Table 19. Prevalence of seed-borne fungi of some selected hybrid seeds collected from Momin Beej Ghar (Agar plate method)

Treatment	% of seed-borne infection			
	<i>Alternaria alternata</i>	<i>Penicillium</i> sp	<i>Fusarium semitectum</i>	<i>A. flavus</i>
African marigold	3.25 a	1.88 b	0.13	0.75 c
Cockscomb	1.25 b	0.00 c	0.13	2.75 a
Petunia	0.00 c	1.75 b	0.00	1.38 bc
Portulaca	0.00 c	2.63 a	0.00	1.63 b
LSD Value (P=0.05)	0.565	0.677	NS	0.663
CV (%)	3.43	4.30	2.94	4.29

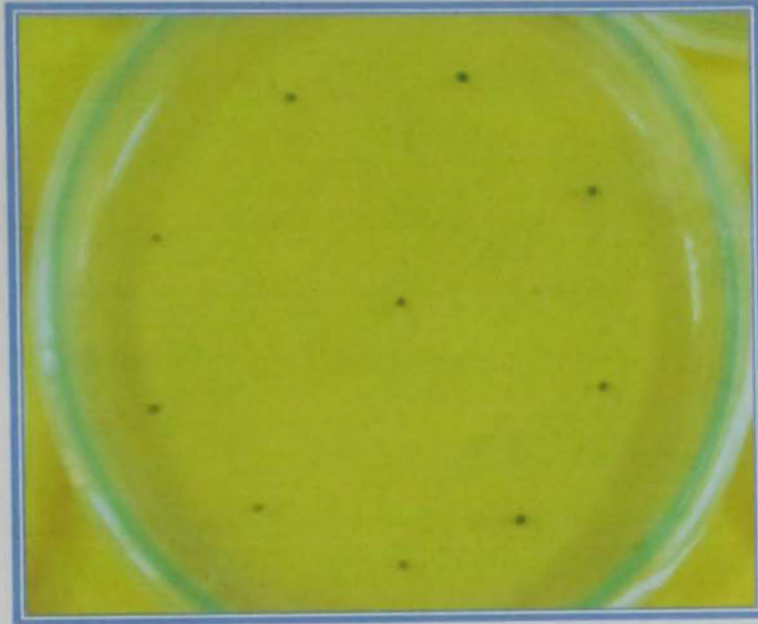


A



B

Plate. 11. A. Petunia seeds were placed on agar plate
B. Fungal colonies derived from infected seeds



A



B

Plate. 12. A. Celosia seeds were placed on agar plate
B. Fungal colonies derived from infected seeds

4.3.2.6 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 20). It was observed that incase of pathogenic infection in cosmos, marigold, calendula and periwinkle seeds the Horticulture garden's seed was better than the others. But according to flower's, periwinkle was the lowest (7.70%) affected seed and marigold was the highest (29.95%) affected seed.

Table 20. Prevalence of total seed-borne fungal infection in local flower seed collected from different locations

Treatment (Flower)	% Total seed-borne infection								
	Blotter method			Agar plate method			Average		
	Arboriculture garden	Ramna park garden	Horticulture garden	Arboriculture garden	Ramna park garden	Horticulture garden	Arboriculture garden	Ramna park garden	Horticulture garden
Cosmos	30.64 b	28.64 c	18.39 c	22.75 a	22.26 a	24.25 a	26.69 b	25.45 b	21.32 b
Marigold	48.85 a	41.77 a	45.51 a	18.38 b	21.13 b	22.38 b	33.62 a	31.45 a	29.95 a
Calendula	32.39 b	36.27 b	24.64 b	13.20 c	10.70 c	9.63 c	22.80 c	23.52 c	17.13 c
Periwinkle	10.27 c	13.26 d	16.14 c	7.14 d	7.13 d	7.25 d	8.71 d	10.20 d	7.70 d
LSD Value (P=0.05)	4.632	4.184	3.593	2.165	1.011	1.242	2.289	1.386	2.288
CV (%)	3.36	3.11	2.29	3.24	2.29	3.50	3.58	3.45	4.12



4.3.2.7 Total infection in hybrid flower seed

Significant variation was observed in case of total pathogenic infection in hybrid seeds (Table 21). It was observed that the lowest fungal infection (2.82%) was in portulaca and petunia seeds, where the highest (5.82%) was in african marigold seeds.

Table 21. Prevalence of percent total seed-borne infection in hybrid flower seed

Treatment	% of total seed-borne infection		
	Blotter method	Agar plate method	Average
African marigold	4.13 a	7.51 a	5.82 a
Cockscomb	3.77 b	4.13 b	3.95 b
Petunia	2.51 c	3.13 c	2.82 c
Portulaca	1.38 d	4.26 b	2.82 c
LSD Value (P=0.05)	0.984	0.946	1.115
CV (%)	3.11	3.38	3.18

Chapter 5

Discussion



CHAPTER 5

DISCUSSION

The experiment was conducted with two types of flower seeds viz. (1) Four local flower seeds (cosmos, marigold, calendula and periwinkle) and each of the flower seeds was collected from three different location (Arboriculture garden, Ramna park garden and Horticulture garden) of Dhaka district and (2) Four hybrid flower seeds (african marigold, cockscomb, petunia and portulaca) and all hybrid seeds were collected from Momin Beej Ghar, Dhaka district.

The results obtained with dry inspection showed that the purity of different flower seeds collected from different location was acceptable because of percent purity (97.00% - 91.00%) of seeds in every cases was in desired value. This was also true incasing of the presence of percent inert matter (5.50% - 1.50%) and presence of other crop seeds (1.00% - 3.50%).

In the experiment, the germination of seeds of different local flowers collected from different location and different hybrid seeds were determined 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 Horticulture garden, marigold collected from Arboriculture garden and calendula and periwinkle collected from Ramna park garden represented the highest germination 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. Shroti *et*

al. (1985) obtained 20 pathogenic fungi in calendula seeds and found significant reductions in seed germination due to the presence of fungi with the seeds.

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 (Hossain *et al.* 1977, Barma and Fakir, 1981 and Fakir and Halder, 1993).

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 detected in different seeds of local and hybrid flower seeds.

In Blotter method, the total pathogen for cosmos, marigold, calendula and periwinkle ranged between 18.39%–30.64%, 41.77%–48.85%, 24.64–36.27% and 10.27%–16.14%, respectively according to different location (Arboriculture garden, Ramna park garden and Horticulture garden of Dhaka district). Again, it was also observed that fungal incidence was higher in marigold and periwinkle seeds compared to cosmos and calendula considering 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, the highest fungal incidence was recorded in marigold seeds and the lowest in periwinkle seeds. Similar phenomenon was not represented by Agar plate method. The total pathogen for cosmos, marigold,

calendula and periwinkle ranged between 24.25%–22.26%, 22.38%–18.38%, 9.63–13.20% and 7.13%–7.25%, respectively according to different location (Arboriculture garden, Ramna park garden and Horticulture garden of Dhaka district). But among local flower seeds, cosmos had the highest pathogen prevalence and periwinkle had the lowest. It was resulted that periwinkle showed the lowest pathogen prevalence among four local flower seeds by the both Blotter and Agar plate methods.

In case of four hybrid flower seeds (african marigold, celosia, petunia and portulaca) the incidence of six pathogens (*Alternaria alternata*, *Penicillium* sp, *Fusarium semitectum*, *Aspergillus niger*, *Aspergillus flavus* and *Rhizopus* sp) were comparatively very much lower than that of blotter test. But among the four hybrid flower seeds, african marigold showed the highest incidence of fungi where portulaca and petunia showed the lowest. These four hybrid flower seeds were collected from one location but incidence varied due to different types of flowers.

The fundings 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. 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 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, specially the pathogenic ones in flower seeds.



Chapter 6

Summary and Conclusion



CHAPTER 6

SUMMARY AND CONCLUSION

The experiment was conducted at the Seed Health Laboratory of the Department of Plant Pathology of Sher-e-Bangla Agricultural University (SAU) during the period from January, 2008 to March, 2009 to study the prevalence of fungi associated with seeds of some selected flowers.

The experiment was conducted with two types of flower seeds viz. (1) Four local flower seeds (cockscomb, marigold, calendula and periwinkle) and each of the flower seeds was collected from three different location (Arboriculture garden, Ramna park garden and Horticulture garden) of Dhaka district and (2) Four hybrid flower seeds (african marigold, cockscomb, petunia and portulaca) and all hybrid seeds were collected from Momin Beej Ghar, Dhaka district.

During the experiment the data were collected on dry inspection of seeds (percent pure seeds, inert matter and seeds of other crops), germination percentage and fungal incidence for the both of local and hybrid seeds. Percent seed borne fungi was determined by blotter method and agar plate method individually but for the germination test only blotter method was used.

The results showed that the highest percent (%) of pure seeds of local cockscomb, marigold, calendula and periwinkle was comparatively high and there was no considerable variation among seeds of local varieties collected from all the seed sources. This result was also valid for percent inert matter and presence of other crop seeds.

Percent seed germination by blotter method varied significantly for the same variety collected from different locations. The germination percentage of marigold (95.25%) from Arboriculture garden seed was highest. But in case of hybrid seeds collected from Momin Beej Ghar the highest germination was in african marigold seed (98.25%) compared to cockscomb, petunia and portulaca seed.

The lowest infestation of *Alternaria alternata* for calendula (1.88%) was in Horticulture garden seed but the highest for marigold (40.97%) were in Arboriculture garden seed. The lowest infestation of *Penicillium* sp for cosmos (0.25%) was in Ramna park garden seed but the highest for marigold (2.88%) was in Ramna park garden seed. The lowest infestation of *Fusarium semitectum* for periwinkle (0.63%) was in Arboriculture garden seed but the highest for calendula (22.75%) were in Arboriculture garden seed. The lowest infestation of *Aspergillus niger* for marigold (0.88%) was recorded in Ramna park garden and Horticulture garden seed but the highest for calendula (8.25%) were in Horticulture garden seed. The lowest infestation of *Aspergillus flavus* for calendula (0.38%) was in Arboriculture garden seed but the highest for periwinkle (8.88%) were found in Horticulture garden seed. The lowest infestation of *Rhizopus* sp for periwinkle (0.38%) was in Arboriculture garden seed but the highest (1.5%) were recorded in Horticulture garden periwinkle seed.

The incidence of *Penicillium* sp, *Fusarium semitectum*, *Aspergillus niger* and *Rhizopus* sp on hybrid seeds were insignificant but for *Alternaria alternata* and *Aspergillus flavus* were significant. The lowest infestation of *Alternaria alternata* (0.25%) and *Aspergillus flavus* (0.25%) were recorded

in portulaca but the highest infestation (1.75% and 1% respectively) was in african marigold seed.

In Agar plate method, the lowest infestation of *Alternaria alternata* for calendula (0.38%) was recorded in Horticulture garden seed but the highest for marigold (21.63%) was in Horticulture garden seeds. The lowest infestation of *Fusarium semitectum* for marigold (0.38%) was in Arboriculture garden seed but the highest for calendula (11.63%) was in Arboriculture garden seed. The lowest infestation of *Aspergillus niger* for periwinkle (0.38%) was in Ramna park garden seed but the highest for periwinkle (8.75%) was in Arboriculture garden seed.

The incidence of *Fusarium semitectum* on hybrid seeds was insignificant but for *Alternaria alternata*, *Penicillium* sp, and *Aspergillus flavus* were significant. The lowest infestation of *Alternaria alternata* (1.25%), *Penicillium* sp (1.75%) and *Aspergillus flavus* (0.75%) were recorded in cockscomb, petunia, portulaca and african marigold seeds respectively, but the highest infestation (3.25%, 2.63% and 2.75%) of this fungi were in african marigold, portulaca and cockscomb seeds respectively.

From the result obtained in the experiment it can be concluded that incase of seed-borne fungal incidence of cosmos, marigold, calendula and periwinkle seeds, the Horticulture garden's seed was better than the others. But in respect of flower's, periwinkle seed infection was the lowest and marigold seed infection was the highest affected seed. But incase of hybrid flower seeds, the lowest fungal infection was in portulaca and petunia seeds, where the highest was in african marigold seeds.



Chapter 7

Literature Cited



CHAPTER 7

LITERATURE CITED

- Barma, A. C. and G. A. Fakir. 1981. Prevalence and pathogenicity of fungi associated with the seeds of kaon (*Seteria italica*). Bangladesh J. Agri. 6(2-3): 6-14.
- Chese, A.R. 1998. Alternaria Diseases of Ornamentals. Western Farm Service. Chase Res. Gardens, Inc. 3142 Squirrel Hollow. Mt. Aukum, CA. 5656-0168.
- Chou, J. K. and W. S. Wu. 1995. Seed-borne fungal pathogens of ornamental flowering plants. Seed Sci. and Technol. 23(1): 201-209.
- Dias, M. R., S. De and M. T. Lucas. 1978. Fungi Lusitaniae. XXVI-Agronomia. Lusitana. 38(4): 285-295.
- Fakir, G. A. 1983. Teaching, Research and Training Activities on Seed Pathology in Bangladesh. Seed Sci. & Technol. 11: 1345-1352.
- Fakir, G. A. and N. Halder. 1993. Seed-borne fungi of chilli. Bangladesh J. P1. Pathol. 7: 34-37.
- Hafiz, M. 2009. Flower production. Bangladesh Web News. February. 1-2pp.
- Hossain, M., G. A. Fakir and A. Momin. 1977. Distribution of some seed-borne pathogens of rice. Bangladesh J. Agri. Sci. 4: 179-181.
- ISTA, 1999. International Rulers for Seed Testing Association. Seed Sci. and Technol. 27, Supplement, 333pp.
- ISTA, 2005. International Rulers for Seed Testing Association. Seed Sci. and Technol. 27, Supplement, 333pp.

- Javaid A, Bajwa R, Shafique S, Javaid A, Jabeen K, Shafique S. 2008. Fungistatic activity of aqueous and organic solvent extracts of rice varieties on phytopathogenic fungi. *Allelopathy J.*, 22: 363-370.
- Keisuke tomioka, Toyozo Sato and Hiroki koganezawa. 1998. Marigold leaf spot caused by *Alternaria tagetica* new to Japan. *J. Gen Plant pathol* 66: 294-299.
- Karlatti, R. S. and P. C. Hiremath. 1989. Seed-borne nature of leaf and inflorescence blight in marigold and its host range. Department of Plant Pathology, University of Agricultural Scinces, Bangalore. 18(12): 180-181.
- Lim, G. 1969. Fusarium wilt of Marigolds. *Mycol. Appl*, 39(3-4): 345-348
- Mathur, S. B. and O. Kongsdal. 2003. Common Laboratory Seed Health Testing Methods for Detecting Fungi. Danish Govt. Institute of Seed Pathology for Devloping Countries, Copenhagen, Denmark Published by ISTA, Switzerland. 425pp.
- Nath, R., Mathur, S. B and Neergaard, P. 1970. Identification of *Fusarium* sp on seeds as they occur in blotter test. Proceeding of the International Seed Testing Association. 35: 121-144.
- Neergard, P. 1979. Seed Pathology. The Macmillan Press Ltd. 1: 839pp.
- Noor, R. 2010. Ful Neba Ga Ful. The Prothom Alo. January. 1-2pp.
- Pape, H. 1942. "Die Alternaria krankheit der zinnia and ihre Bekampfung" *Angew. Bot.* 24: 61.
- Prione, P. P. 1960. Disease and pests of ornamental plants. 5th ed. Constable and Co. Ltd. London. 537pp.
- Prasad, B. K. 1987. Pre-harvest Endophytic seed mycoflora of garden plants belonging to Asteraceae. *Indian J. Mycol. and Pl. Pathol.* 17(3): 339pp.
- Richardson, M. J. 1990. An Annotated list of seed-borne diseases. 4th ed. The International Seed Testing Association, Switzerland. 338pp.

- Saksena, H. K. and B. B. Singh. 1959. The effect of seed borne pathogen of Marigold. Plant disease. Repr. 43: 670-673.
- Shrotri, S. C., J. S. Gupta, and R.N. Srivastava. 1985. Seed-borne fungi *Calendula officinalis* and their control. India Phytopath. 38(1): 42-143.
- Srivastava, R. N. and J. S. Gupta. 1983. Pathogenicity of fungi associated with zinnia seeds. Indian Phytopath. 36: 14-16.
- Sultana, S. 2004. Prevalence and pathogenicity of fungi associated with the seeds of some selected flowering plants. M. Sc. Ag. Thesis. Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. pp 1-8.
- Talukder, M. J. 1974. Plant Disease in Bangladesh. Bangladesh J. Agri. Res., 1(1); 1-78.
- USDA. 1960. Index of Plant Disease in the United States. Agricultural hand book. No. 165: 531pp.
- Wu, W. S., H. H Chou., S. M. Lin and H. C. Wu. 2001. The effect of seed-borne pathogens on emergence of globe amaranth, calendula and tagetes and the methods of control. J. Phytopath. 149(2): 91-96.
- Wu. W. S., Y. L. Li and H. C. Wu. 1996. Seed-borne fungi of ornamental flower plants. Australasian Plant Pathology 35(3): 373-375.

