I-13/14 STUDY ON HEALTH STATUS OF OKRA SEEDS COLLECTED FROM DIFFERENT LOCATIONS OF BANGLADESH

DHARMADASH SARKAR

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DECEMBER, 2010



STUDY ON HEALTH STATUS OF OKRA SEEDS COLLECTED FROM DIFFERENT LOCATIONS OF BANGLADESH

BY

DHARMADASH SARKAR

REG. NO. 05-01776

A Thesis

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Approved by:

(Dr. M. Salahuddin M. Chowdhury) Professor Supervisor

Mrs. N. Akhtar.

(Mrs. Nasim Akhtar) Professor Co-supervisor

(Nažneen Sultana) Chairman Examination Committee



Dr. M. Salahuddin M. Chowdhury Professor Department of Plant Pathology Sher-e-Bangla Agricultural University Dhaka-1207, Bangladesh

Mob: +8801713382588 E-mail: smc_1968@yahoo.com

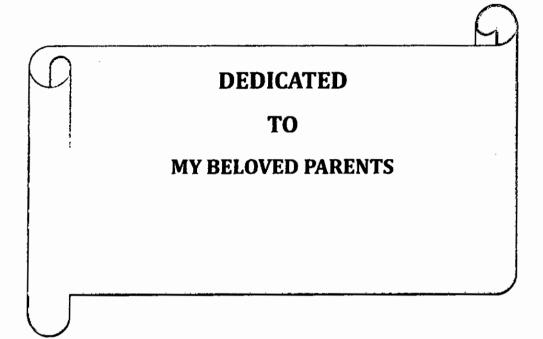
CERTIFICATE

This is to certify that thesis entitled, "Study on Health Status of Okra Seeds Collected from Different Locations of Bangladesh." submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfilment 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 Dharmadash Sarkat, Registration No. 05-01776 under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: December, 2010 Place: Dhaka, Bangladesh (Dr. M. Salahuddin M. Chowdhury) Professor Supervisor







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December, 2010 SAU, Dhaka The Author

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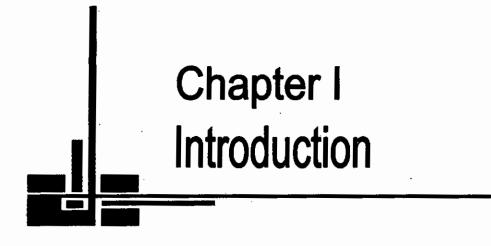
LIST OF SYMBOLS AND ABBREVIATIONS

FULL WORDS	ABBREVIATION
Percentage	%
Cultivar	cv.
Ton	t
Hector	ha
Exempli gratia (by way of example)	e.g.
and others (at ell)	et al.
Species	Spp.
Centimeter	cm
Metric ton	Mt
Bangladesh Agriculture Research Institute	BARI
Sher-e-Bangla Agricultural University	SAU
Journal	J.
Number	No.
variety	var.
Namely	viz.
Degrees of freedom	df.
Form species	f. sp.
United States Department of Agriculture	USDA
International Seed Testing Association	ISTA
United Nations Development Program	UNDP
Food and Agricultural Organization	FAO
Department of Agricultural Extension	DAE
At the rate of	@
Milliliter	ml
Etcetera	etc.
Potato Dextrose Agar media	PDA
Degree Celsius	°C
Gram	g
Bangladesh Bureau of Statistics	BBS
Analysis of variances	ANOVA
Kilogram	Kg
Bangladesh Institute of Nuclear Agriculture	BINA
Bangabandhu Sheikh Mujibur Rahman Agricultural University	BSMRAU
Bangladesh Agricultural University	BAU
Percentages of Co-efficient of Variance	CV%
Least Significant Difference	LSD
Science	Sci.

STUDY ON HEALTH STATUS OF OKRA SEEDS COLLECTED FROM DIFFERENT LOCATIONS OF BANGLADESH

ABSTRACT

Quality and health status of okra seeds collected from farmers, retailers and other from research stations were determined. Total fifty seed samples were collected from nine districts of Bangladesh. Of them, 28 samples were obtained from famers and 17 from retailers and 5 samples from research stations. The samples belonged to 3 varieties viz. Local-1, Local-2 and BARI Dherosh-1. Seed health and quality analysis revealed that moisture content of seed samples were varied significantly. Germination percentage of research station' seeds, retailer' seeds and farmer' saved seeds was 81.93%, 57.82% and 46.10% respectively. Purity percentage varies from 93.68 to 96.94% in different locations and 95.76 to 94.81% in different varieties. Purity percentage of research station' seeds, retailer' seeds and farmer' saved seeds were 96.94%, 95.82% and 94.86%, respectively. Both vigour index (1585.0) and 1000-seed weight (69.11g) were found higher in research station' seeds than the retailer' seeds and farmer' saved seeds. In seed health study, seven fungi namely (1) Aspergillus flavus, (2) Aspergillus niger, (3) Fusarium spp., (4) Macrophomina phaseolina, (5) Colletotrichum dematium, (6) Rhizopus spp. and (7) Curvularia spp. were found to be associated with the seed samples. Among the fungi prevalence Aspergillus flavus was maximal which was followed by Fusarium spp. All the seven fungal pathogens were more prevalent in farmer' saved seeds compared to other seeds. The seeds were graded as (1) apparently healthy seeds (G-I), (2) discolored seeds (G-II), (3) spotted seeds (G-III) and (4) shriveled & broken seeds (G-IV) based on physical conditions of the seeds. It was found that apparently healthy seeds under G-I yielded lowest incidence of fungi and highest percentage of germination in comparison to other grades.





CHAPTER I INTRODUCTION

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Okra (*Abelmoschus esculentus* L. Moench) is an annual vegetable crop in Bangladesh. Though it is grown round the year, its production is mainly concentrated during summer season. It is locally known as "Dherosh" or "Bhendi" which belongs to the family Malvaceae. It is probably originated from tropical Africa or possibly tropical Asia and widely grown throughout the tropics (Tindall, 1988). The crop is well distributed throughout the Indian subcontinent and East Asia (Rashid, 1999).

Okra is very popular for its high nutritive value and delicacy. It is used for multiple purposes such as to get clean molasses or sugar and raw materials for making paper. It has medicinal value as well (Rashid, 2000). During rainy season, scarcity of vegetable is a serious problem in Bangladesh. Okra contributes an important share to meet the demand of vegetable during that lean period of the year. It is a nutritious vegetable containing 86.1% water, 2.2 % protein, 0.2% fat, 9.7% carbohydrate, 1% fiber and 0.8% ash (BARI, 2010).

In Bangladesh it covers an area of 7,152 hectors with a production of 24, 230 metric tons per year, and its average yield is about 3.38 t/ha in 2009-10 growing season in Bangladesh (BARI, 2010). Yield per unit area is very low

compared to other countries where the yield is as high as 7-12 t/ha (Anon, 1995). Mainly diseases and poor quality seeds are responsible for such low yield in Bangladesh (Mew, 1997).

Seeds are common carrier of plant pathogens, which act as the primary source of inoculants of many diseases (Rahman and Mia, 1998). Contaminated seeds can often result in poor germination and poor seedling vigour, resulting in an unhealthy crop.

Healthy seed is the foundation of healthy plant, a necessary condition for good yield (Diaz et al., 1998). Field fungi associated with seeds cause deterioration of seed quality affect viability and reduce seed germination (Haware 1971, Srivastava and Gupta, 1981). Storage fungi like *Aspergillus, Penicillium, Fusarium* etc. deteriorate seeds in storage (Christensen, 1963).

Most of the farmers in Bangladesh use their own produced and saved seeds without considering seed health status. Moreover, they are not careful enough about quality of seeds.

Many pathogens are associated with the seeds, especially seed-borne fungal pathogens. The most important seed-borne pathogens infecting the okra seeds are *Aspergillus* spp., *Fusarium* spp., *Macrophomina phaseolina*, *Colletotricum* spp., *Curvularia* sp., etc. Among them *M. phasolina* is a common pathogen of many crops and survive in seed as well in soil (Dhingra and Sinclair, 1973).

In okra plant it cause die back and root and collar rot (Goel and Mehrotra, 1973 &1977). The infection of *M. phasolina* individually or along with *Colletotrichum dematium* has been reported in fields of Bangladesh (Fakir and Mrida, 1985). *Fusarium* spp. is the most important seed-borne pathogens of okra.

Seed-borne disease causes enormous loss of crops in Bangladesh. About 200 seed-borne diseases including 100 of major importance occur on 50 different crops grown in the country (Fakir, 1982). In compiling the world list of pathogens of okra, 26 pathogens are reported for different diseases (USDA, 1960), among them 13 pathogens are seed-borne of which 11 are fungi (Richardson, 1979). In Bangladesh okra suffers from a number of different diseases among them 14 are seed-borne of which 6 are major and 8 are minor (Akanda, 1993).

Major seed-borne diseases of okra in Bangladesh are seed rot, seedling blight, die back and anthracnose caused by *Colletotrichum dematium*: stem rot, die back caused by *Macrophomina phaseolina*: seed rot, germination failure and seed discoloration caused by *Aspergillus* spp. and seed rot / seedling blight caused by *Fusarium oxysporium* f. sp. vasinfectum (Fakir, 2001). Major seedborne fungal pathogens i.e *Colletotrichum dematium* and *Macrophomina phaseolina* are both seed transmitted. Fakir *et al.* (1977) reported that the prevalence of both the pathogens depending on the seed sources is 32% and 48.8%, respectively. Besides, seed-borne pathogens also play an important role in deteriorating the quality of crops by transmitting the disease in the field. So, for profitable and healthy crop production healthy seeds are urgently needed.

However, the germination, yield and quality of okra seeds depend on their inherent quality. Their inherent quality cannot be assessed easily just from their external appearances. Genetic and physical purity, germination capacity, moisture content, vigour and health status are the important quality parameters of a good seed (Kant *et al.*, 1999).

Okra seeds which are available in the local market are usually of low quality and are mostly collected from indigenous, adulterated and degenerated varieties (Anon., 1995). The growers use such low quality seeds (Vossen, 1994.) and get poor yield.

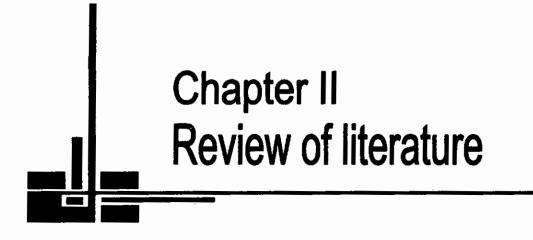
From the forgoing deliberations, it appears that seed-borne diseases are responsible for low yield and production of poor quality okra seeds in Bangladesh. But the health status of okra seeds produced by the farmers in the country is not preciously known. But successful okra cultivation largely depends on the use of quality healthy seeds.

The national seed policy of Bangladesh seeks to make the best quality seeds of improved varieties of crops conveniently and efficiently available to farmers.

To assure planting of best quality seed, an assessment of present health status and different quality attributes of okra seeds being used by farmers is necessary.

Considering the above facts, the present investigation was conducted with following objectives:

- To find out quality and health status of okra seeds collected from different locations of Bangladesh.
- To determine the incidence of seed-borne fungi and germination percentage in different graded okra seeds.





CHAPTER II

REVIEW AND LITERATURE

Very little work has been done on the effect of seed-borne fungal diseases of okra. Therefore, effort has been made to review the literature on the prevalence of seed-borne diseases of okra, seed-borne fungi and its pathogenic effect on the crop.

2.1. Fungal pathogens associated with okra seeds

Fakir (1980) listed four important seed-borne fungal pathogens in okra namely, Aspergillus spp., Colletotrichum dematium, Fusarium spp. and Macrophomina phaseolina in Bangladesh.

Khanzada *et al.* (1988) and Gupta *et al.* (1989) reported that the whole and sectioned dry *Abelmoschus esculentus* seeds showed fungal infection on the surface and in the interior. Some seed components were discolored. Sixteen fungi were isolated from germinating seeds. While only 4 were re-isolated from seedlings (Adisa and Aborisade 1988). However, some workers also reported that the okra seeds may carry large number of seed-borne fungi which reduce seed germination because of seed rot or seedling mortality.

Gupta *et al.* (1989) investigated fungi associated with 9 samples of okra seeds collected from different areas in Meerat, Uttar pradesh, India by blotter and agar plate methods. The percentage of damaged, deformed and discolored seeds varied in seeds collected from different places. *A. alternate, Curvularia*

lunata and A. flavus the major fungi associated with discolored seeds. After seed surface sterilization A. alternata, A. fiavus, A. niger, C. lunatus, Fusarium spp., were recorded as internally seed-borne and caused seedling mortalities.

Hema *et al.* (1989) reported that okra crop has been infected by many fungi, a majority of which cause fruit disease at maturity leading to seed infection.

Fernandes et al. (1992) isolated Ascochyta abelmoschi, Botryodiplodia theobromae, Colletotrichum sp., Fusarium oxysporum, Macrophomina phaseolina, Phomopsis sp., Rhizoctonia solani, Aspergillus sp., Curvularia sp., Penicillum sp., Rhizopus sp., Tricoderma sp., etc from okra seeds produced in Rio de Janeiro state, Brazil using blotter method and by plating on PDA. Pathogenecity was confirmed for the first 7 species.

Islam, (1997) identified seven seed-borne fungi from seed sample of 'IPSA OKRA' by the standard blotter method. These were Alternaria, Ceratocystis, Chaetomium, Cladosporium, Curvularia, Fusarium and Nigrospora.

Pun *et al.* (1998) collected a total of 10 okra seed samples from different locations in Tamil Nadu, India *M. phaseolina* was associated with all the seed samples and it was shown that infection led to both pre and post emergence mortality which confirmed the seed to seedling transmission of the pathogen.

Prasad et al. (2000) isolated twenty fungal species from stored okra seeds. The dominant fungal species were A. niger, A. flavus, A. sydowii, A. candidus, F. monil [fujikuroi], A. alternata, Curvularia lunata [Cochliobolus lunatus], Penicilijum oxaticum and Mucor sp.

Rashid (2000) listed six important fungal pathogens of okra in Bangladesh. They were Cercospora spp., Alternaria spp., Phyllostica sp., Colletotrichum dernatium, Ascochyta abelmoschi, Choanephora cucurb itarum, Rhizocronia sp., M. phaseolina, Fusarium spp.

Shahid *et al.* (2001) studied on the survival of seed-borne inoculums and seed transmission of *M. phaseolina* was studied in 5 okra seed samples collected from different locations in Kanpur, Uttar Pradesh India. The sample showing the highest count of *M. phaseolina* was used for further investigations. The fungus was found to be present in the seed coat and embryo in 45 and 20%, respectively. Seed transmission of the fungus on okra cv. Pusa Makhmali seedling was tested using the roll towel, water agar and growing on test. All methods showed that seed infection due to *M. phaseolina* led to both pre and post emergence mortality of okra, confirming seed to seedling transmission of the pathogen.

Alam and Bazlur Rashid (2005) listed eight fungi such as *Aspergillus* spp., *Penicillium* sp., *Curvularia* sp., *Fusarium* spp., *Rhizopus* sp., *Colletotrichum* sp., *Alternaria* sp. and *Macrophomina* sp. associated with the seed samples of okra available in the market.

Fakir (2000) listed 14 different fungi representing 6 genera in okra seeds in Bangladesh. The genera were Aspergillus, Cercospora, Fusarium, Colletotrichum, Macrophomina and Penicillium. The listed fungi were three species of Aspergillus namely, Aspergillus flaviis LK, Aspergillus repens De Bary, Aspergillus ruber T&C, three species of Colletotrichum namely, Colletotrichum dematium (Pers. Ex. Fr.) Grove, C. gloeosporioides Penz and C. gramminicola and five species of Fusarium namely, Fusarium equiseti (Corda) Sacc, F. moniliforme Sheldon, F. oxysporum f.sp. vasinfectnm, F. semitectum Berk and Rave and F. solani (Mert.) Sacc.

Bhattiprolu and Rahman (2008) evaluated twelve okra entries in Hyderabad, Andhra Pradesh, India. Performance of different entries over three years revealed that disease reduction in all the entries was significantly higher than in the control. Mean yield ranged from 47.93 q/ha in KS-410 to 80.93 q/ha in HIGH-068. HIGH-068 recorded the maximum yield increase of 55.28%, followed by Arka Abhaya (45.52%), VRO-6 (37.74%) and VRO-5 (36.91%). Disease reduction ranged from 84.75% in Arka Anamika to 95.17% in VRO-4. However, resistance was not correlated with increase in yield. High-yielding entry, HIGH-068, recorded 91.54% reduction in disease compared to VRO-4 with 95.17% disease reduction but only 6.41% increase in yield.

Begum (2005) reported that *Macrophomina phaseolina* and *Furasium verticilloides* cause collar-rot, seedling-rot and other severe diseases at fruit maturing stages of okra. These stages were located in all the components of the

seeds. The seeds collected from seeds infected with *Macrophomina phaseolina* and *Fusarium verticilloides* revealed 100% infection. Such seeds resulted in pre- and post-emergence mortalities. Inoculated seeds also showed pre- and post-emergence death of the seedlings. *Fusarium verticilloides* causes the wilt and *Macrophomina phaseolina* causes the collar-rot.

Alam (2002) studied the health of onion, tomato, brinjal, chilli and okra seeds collected from different sources. He observed *Aspergillns flavus*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Penicillhim* sp. and *Rhizopus* sp. as seed borne in case of okra seeds. He found that the prevalence of the pathogen was different on seeds of different sources.

Jamadar et al. (2001) reported the effect of seed mycoflora and nematodes on the colour graded okra in India. They found 27 fungi were associated with the different coloured seeds, among which Aspergillus flavus, Aspergillus niger, Colletotrichum gloeosporides, Fusarium moniliforme, Rhizoctonia solani, Rhizopus spp. and Phomopsis sp. were predominant.

Anam (2000) conducted an experiment on the role of seed-borne fungal pathogen in developing particular disease of okra. He observed different fungal pathogens which were responsible for causing disease in okra. The pathogens were *Botryodiplodia theobrome*, *Cercospora abelmosche*, *Colletotrichum dernatium*, *Corynespora cassicola*, *Fusarium* spp. and *Macrophomina phaseolina*. Ashrafuzzaman (1991) described *Macrophomina phaseolina* and *Fusarium* oxysporum f. vasinfectum as the causal organism of stem rot and wilt diseases respectively of okra. He also reported leaf spots of okra caused by number of fungi, like species of Alternaria, Cercospora and Phyllosticta.

Richardson (1990) listed seven seed borne fungal pathogens on okra (Hibiscus esculentus). The seed-borne pathogenic fungi listed were Ascochyta abelmosche, Choanephora cucurbitirum, Colletotrichum dematium, Fusarium oxvsporum, F. solani, Fusarium spp. and Macrophomina phaseolina.

Bazlur Rashid et al. (1983) reported that Alternaria tennis (Alternaria alternate), Colletotrichum dematiiim, Curviilaria lunata, Fusarium equiseti, Fusarium semitectum and Macrophomina phaseolina were associated in okra seeds where Curvularia lunata was most prevalent followed by Fusarium equiseti.

Sing et al. (1983) identified Abelmoschus esculentus as a new host for the anthracnose and die-back pathogen, Colletotrichum dematium in India.

Fakir (1982) detected seed rot caused by Aspergillus spp., anthracnose caused by Colletotrichum dematium, seed rot/seedling blight caused by Fusarium oxysporum f.sp. vasinfectum and Fusarium solani and stem rot caused by Macrophomina phaseolina as major seed-borne diseases of okra in Bangladesh.

Narayana (1978) detected that seedling blight of okra caused by Rhizoctonia solani and Macrophomina phaseolina, Fusarium blight caused by Fusarium

spp. and pod spot of okra caused by Ascochyta abelmosche were seed-borne disease.

Huan and Jamil (1975) found that *Rhizoctonia* spp. and *Choanephora* sp. were seed-borne caused fruit rot of okra and estimated crop loss was 20% in a diseased plot.

Lambat *el. al.* (1974) reported that *Colletotrichum dematium* was the causal agent of a severe die-back of okra in India. The fungus was found to be seed-borne in the crop.

Cheema and Jhoram (1954) reported that anthracnose disease of okra is caused by a number of seed-borne fungal pathogens. The most predominating fungi were *Colletotrichum* spp. which was influenced by environment and host factors like temperature, relative humidity, sunshine hours and the age of host.

Lambat *et al.* (1974) reported that *Colletotricum demiatium* was the causal agent of a severe die-back of okra in India. The fungus was found to be seed-borne in the crop.

Rangaswami (1979) described two species of Cercospora viz., C. malayensis and C. abelmoschi causing leaf spot disease of vendi (okra) in India.

Sing et al. (1983) identified Abelmoschus esculentus as a new host for the anthracnose and die-back pathogen, Collelotricum demtium in India.

Fakir and Mridha (1985) found Colletotrichum dematium and Macrophomina phaseolina to cause die-back a new disease of lady's finger (okra) in Bangladesh. They reported the pathogens as seed-borne.

Ribeiro *et al.* (1971) reported a new special fungi *Fusarium solani* as the cause of pre and post-emergence rot of okra and this new seed-borne disease occurred widely in organic soils. The pathogen was detected as *Fusarium solani* f. sp. *hibisci*. Chidambaram and Mathur (1975) found *Macrophomina* spp. on the seeds of okra crop in India.

Esuruso et al. (1975) tested freshly harvested seed from 67 cvs. of okra in Nigeria. They found *Fusarium moniliforme* in all samples, *F. semitectum* and *F. solani* with 87% and 30% seeds, respectively with many other probably non-pathogenic fungi.

Fakir et al. (1977) reported that Macrophomma phaseolina and Colletotrichum dematium were found in okra seeds and these two fungi were responsible for germination failure, pre and post-emergence damping off of the seedlings and also capable of causing infection to the older plants of the crop. Their studies also indicated that the two pathogens became seed-borne through secondary infection. Transmission of both the pathogen through okra seeds was the first record in Bangladesh. They also reported about unpublished data at the Danish Governmental Institute of Seed Pathology for Developing Countries, Denmark, that Colletotrichum dematium occurred on okra seeds collected from Nigeria and Macrophomina phaseolina from Ghana, India and Nigeria.

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Gangopadhyay and Kapoor (1977) traced *Fusarium oxysporum* f. sp. *vasinfectum* on *Hibiscus esculentus* as seed-borne and reported loss in dry weight of infected seeds from 20.3-24.2% due to this pathogen.

Alam (2001) recorded Alternaria, Aspergillus, Penicillium, Curvularia and Rhizopus from tomato seeds; Aspergillus, Penicillium, Curvularia, Fusarium, Phomopsis and Rhizopus from brinjal seeds. Aspergillus, Penicillium and Rhizopus from onion seeds and Aspergillus, Penicillium, Curvularia, Fusarium, Rhizopus, Collectotrichum, Alternaria and Macrophomina from Okra seeds in blotter method.

Gupta and Chowdhury (1995) isolated Alternia, Aspergillus, Chaetomium, Colleclrichum, Curvularia, Drechslera, Fusarium, Mucor, Penicillium and Rhizopus from seeds of bhindi (okra), brinjal and chillies.

Moretto et al. (1977) reported that pathogenic fungi such as Colletotrichum spp., Rhizoctonia solani, Fusarium spp. and Alternaria spp. were associated with okra seeds.

Robbs *et al.* (1977) noted the occurrence of Fusariosis of okra in the Baixada Carioca, Brazil and reported *Fusarium oxysporum* f. sp. *vasinfectum* as the cause of wilt of okra. The pathogen was seed transmitted and advised to use certified seeds for plantation.

Fakir (1986) identified 17 different fungi representing 12 genera in okra as seed-borne collected from three districts viz. Bogra, Mymensingh and

Rajshahi. He reported the identified genera as Aspergillns, Alternaria, Colletotrichum, Corynespora, Chaetomium, Curvularia, Doratomyces, Epicoccum, Fusarium, Penicillium, Macrophomina and Rhizopus and listed most prevalent fungi in order of prevalence were Aspergillus flavus, Chaetomium globosum, Penicillium spp., Aspergillus niger, Aspergillus sydowii, Curvularia lunata, Fusarium spp. and Doratomyces spp.

Fakir (1987) again recorded 15 different fungi representing 10 genera in okra seeds collected from three districts viz. Bogra, Mymensingh and Rajshahi. The genera of fungi were Aspergillus, Alternaria, Colletotrichum, Chaetomium, Curvularia, Epicoccum, Fnsarium, Penicillium, Macrophomina and Rhizopus. The most commonly occurring fungi in order of prevalence were Aspergillus flavus, Penicillium spp., Fusarium spp. (F. equiseti, F. oxysporum, F. semitectum and F. moniliforme), Aspergillus sydowii, Aspergillus niger, Alternaria alternata, Colletotrichum dematium, Macrophomina phaseolina, Curvularia lunata and Rhizopus nigricans.

Gupta et al. (1989) investigated fungi associated with 9 samples of okra seeds collected from areas in Meerut, Uttar Pradesh, India by blotter and agar plate method. They found that Alternaria alternata, Curvularia lunata and Aspergillus flavus were the major fungi associated with discolored seeds. They also recorded Alternaria lunata, Aspergillus flavus, Aspergillus niger, Cochliobolus lunata, Fusarium semitectum, (F. pallidoroseum) and F. moniliforme (Gibberella fujikuroi) from surface sterilized sevendhari and pusa

savvani okra seed as internally seed-borne Which produced varying degrees of seed and seedling mortality.

Majid (1996) identified 9 different fungi representing 7 genera in okra seeds. Aspergillus spp., Alternaria spp., Colletotrichum dematium, Fusarium spp., Macrophomina phaseolina, Penicillium spp. and Rhizopus spp. were detected as the fungi from the collected seed samples of Thakurgaon and Mymensingh Sadar upazilla.

Quayum (1999) identified and reported 10 seed-borne fungal pathogens in okra seeds collected at three-harvested time from four locations under Mymensingh Sadar upazilla. The predominant ones, in order to prevalence, were *Fusarium* oxysporum, *F. moniliforme*, Macrophomina phaseolina, Aspergillus flavus and Colletotrichum dematium. Highest seed-borne fungal infections were recorded at the harvest time, i.e. 1 to 10 August. This was suggested as the most unsuitable time for collection of okra seeds.

Atia and Tohamy (2004) grown okra plants in Eltal-Elkabeer (El-Baalwa village), Ismailia governorate, Egypt, on sandy soil under drip irrigation systems. He found typical symptoms of *Alternaria* leaf spot symptoms on leaves started as light brown spots that became concentric brown spots varying in size. The spots spread to cover large areas of the infected leaves. In case of severe infection, infected leaves eventually wilted. The causal organism was identified as *Alternaria alternata* based on cultural characteristics and light

microscopic analysis. This is thought to be the first record of *Alternaria* leaf spot on okra in Egypt.

Henz *et al.* (2007) conducted a study to characterize the causal agent, to fulfill Koch's postulates, and to determine some conditions conducive to the disease in Brazil. The pathogen was identified as *Rhizoctonia solani* based on morphological characteristics. The pathogen was able to grow in different materials used for assembling crates and packs of horticultural products, such as pinewood, corrugated carton, plastic, styrofoam and newspaper sheets when kept in humid chambers (24 degrees C, 96% RH). The disease occurrence can be related to careless handling practices and to the transmission of *R. solani* propagules by infected plant debris or soil particles. This is thought to be the first report of *R. solani* causing postharvest rot in okra pods in Brazil.

2.2. Location of fungal pathogens

Agrawal and Singh (2000) observed that infected seeds of okra (*Abelmoschus* esculentus) with *M. phaseolina* appear brown to black and show die-back, root and collar rot diseases. The incidence of the disease in seed samples collected from Rajasthan, India during 1996-99 varied from 0.5-18%. Infected seeds were symptomatic with or without micro-sclerotia. In asymptomatic seeds, the mycelium was confined to the seed coat and endosperm only, whereas mycelium and micro-sclerotia occurred in the seed coat, endosperm and embryo of symptomatic seeds. Extra-embryonal infection resulted in disease

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transmission to seedlings whereas intra-embryonal infection mostly caused preand post-emergence mortality.

Adisa and Aborisade (1987) studied seed-borne mycoflora of two okra cultivars and their effects on seed quality in Nigeria. They found that whole and sectioned dry *Abelmoschus* (*Hibiscus esculentus*) seeds showed fungal infection on the surface and or the interior parts of the seed. Some seed components were discolored. Sixteen fungi were isolated from germinated seeds, while only four were reisolated from seedling, *Aspergillus flavus*, *Botryodiplodia theobrome* and *Penicillium digitatum* were predominate.

Nassema et al. (1983) observed that storage fungi such as Aspergillus flavus, A. niger and Rhizopus stolonifer were externally as well as internally seed-borne in the seeds of Abelmoschus esculentus.

Neergaard (1979) found many seed borne fungi infecting seed coat causing conspicuous black brown to black necrotic discoloration, germination reduction and germination failure. The best known samples were the effects of *Aspergillus* spp. in tomtato, okra and radish; *Penicillium* spp. in okra and radish, *Alternaria* spp. in radish, *Fusariiim* sp. in onion and *Colletotrichum gloeosporioides* in okra.

Goel and Mehrotra (1973) isolated Rhizoctonia bataticola (Macrophomina phaseolina) from diseased root samples and seeds of Abelmoschus esculentus

and found 30-40% superficial contamination of seeds and 12-22% deep seated infection.

Shahid *et al.* (2001) studied the survival of seed borne inoculums and seed transmission of *M. phaseolina* in 5 okra seed samples collected from different locations in Kanpur, Uttar Pradesh. The sample showing the highest count of *M. phaseolina* was used for further investigations. The fungus was found to the present in the seed coat and embryo in 45% and 20% of cases, respectively. Seed transmission of the fungus on okra cv. Pusa Makhmali seedlings was tested using the roll towel, water agar and growing-on test. All methods showed that seed infection due to *M. phaseolina* led to both pre and post emergence mortality of okra, confirming seed to seedling transmission of the pathogen.

Kumkum Gupta et al. (1989) found that Alternaria alternata, Curvularia lunata and Aspergillus flavus were the major fungi associated with discolored seeds. They also recorded Alternaria lunata, Aspergillus flavus, A. niger, Cochliobolus lunatus, Fusariurn semitectum and F. monilliformae from pusa sawani okra seed as internally seed-borne which produced varying degrees of seed and seedling mortality.



2.3. Seed-borne fungi of okra and their pathogenic effect

Fakir (1976) detected 25 fungi in okra seeds collected from greater Mymensingh district of Bangladesh. Of the fungi encountered, *Aspergillus* spp., *Colletotrichum dematium*, *Fusarium oxysporum* f. sp. *vasinfectum*, *Fusarium solani and Macrophomina_phaseolina* were responsible for causing seed rot, anthracnose, seed rot/ seedling blight and stem rot, respectively.

Fakir (1982) detected seed rot caused by *Aspergillus* spp. anthracnose caused by *Colletotrichum dematium* (Pers. ex. Fr.) Grove, seed rot seedling blight caused by *Fusarium oxysporum* sp. vasinfectum (Atk). Synd and Hans and *Fusarium solani* (Mart) Sacc. and stem rot caused by *Macrophomina phaseolina* (Tassi) Gold as major seed-borne diseases of okra in Bangladesh.

Singh et al. (1983) identified Abelmoschus esculentus as a new host for colletotrichum dematium in India.

Fakir (1986) identified 17 different fungi representing 12 genera in okra as seed borne collected from three districts viz. Bogra, Mymensingh and Rajshahi. He reported the identified genera as *Aspergillus*, *Alternaria*, *Colletotricum*, *Corynespora*, *Chaetomium*, *Curvularia*, *Doratomyces*. *Epicoccum*, *Fusarium*, *Penicillium*. *Macrophomina* and *Rhizopus*. According to him, the most prevalent fungi, in order of prevalence, were *Aspergillus flavus*.

Chaetomium globosum, Penicillium spp., Aspergillus niger. Aspergillus sydowii, Curvularia lunata, Fusarium spp. and Doratomyces spp.

Fakir (2000) listed 14 different fungi representing 6 genera in okra seeds in Bangladesh. The genera were Aspergillus, Cercospora, Fusarium, Colletrotrichurn, Macrophomina and Penicillium. The listed fungi were three species of Aspergillus namely, Aspergillus flavus three species of Colletotrichum namely, Colletotrichium dematium (Pers. ex. Fr.) Grove,C gloeosporioides Penz, and C. graminicola (Ces.) Wilsm and five species of Fusaruim namely, Fusarium equiseti (Corda) Sacc, F. moniliforme. Sheldon, F. oxysporium f. sp. vasinfeeturn, F. semitectum Berk and Rave and F. solani (Mert.) Sacc.

Alam (2002) reported that by using blotter method he obtained different pathogens for vegetables seeds. He recorded *Aspergillus*, *penicillium*, *Curvularia*, *Fusarium*, *Rhizopus*, *Colletotrichum*, *Alternaria* and *Macrophomiria* from okra seeds.

2.4. Effects of storage condition on okra seed

Sahoo and Srivastava (2002) conducted an experiment to determine the effect of moisture content (M.C.) on the physical properties of okra seed and they found that the average length, breadth and thickness of the seed varied from 5.92 to 7.30, 4.71 to 5 40 and 4.59 to 5.36 mm, respectively, as the moisture content increased from 8.16 to 87.57%. The roundness and sphericity increased from 77.76 to 79.35% and 74.48 to 76.52% respectively, with an increase in moisture content from 8.16 to 19.56% d.b. and then decreased to 72.39 and 70.63% respectively, with further increase of moisture content.

Kapri *et al.* (2003) conducted a Field experiment to develop a suitable inexpensive method for seed invigoration treatments to improve the germination ability and field performance of okra cv. Pankaj seeds. The seeds were sun dried to a moisture content of 9.5% then stored in rubber stoppered glass bottles until treatment.

2.5. Seed germination

Alam (2002) conducted an experiment to study the germination and /health of some vegetable seeds collected from BADC, BRAC, Local seed company. Local seed trader and farmer. In case of okra seed he obtained highest germination (87%) of BADC seeds and lowest of farmers seeds (70%). For brinjal, highest germination was observed 78% in BADC seeds and lowest 44% in local seed company seeds.

In case of chilli, highest was in farmers seeds (88%) and lowest was in local seed trader seeds (4.6%). He concluded that in respect of germination, BADC seeds both for okra and brinjal and farmers seeds for chilli performed better.

Nema (1986) conducted an experiment to determine a minimum seed, germination standard of certified seeds of vegetable crops for India, which was 65% for okra.

Lorenz and Maynard (1980) conducted an experiment to find out a minimum seed germination standard of vegetables for USA which was 50% for Okra.

2.6. Seedling vigour

Seed vigour may be defined as the natural robustness and active good health in seed, which permits germination to proceed rapidly under a wide range of environmental condition including both favorable and stress condition.

According to Ching (1973) vigour may be defined as the potential for rapid and uniform germination and fast seedling growth under general field conditions.

Woodstock (1969) stated that vigour might be defined as that condition of active good health and natural robustness in seed, which upon planting, permits germination to proceeds rapidly under a wide range of growing conditions.

Thomson (1979) revealed that the germination capacity of a seed lot indicates its ability to establish seedlings in good field conditions. Vigour indicates its ability to do so in stressed conditions. The germination figure may therefore, include seeds of insufficient vigour, which may not be suitable for good establishment on the form.

Sheperd and Winston (1999) in India studied the performance of different okra hybrids (Varsha, Vijaya and Adhunik) including an open pollinated local cultivar (Prabhani Krauti) as control. They observed that the Adhunik was most

suitable for cultivation and Prabhani Kranti performed better than the rest two hybrids.

Agrawal (1996) revealed that seedling dry weight method is an indirect method for vigour test. In this method scedlings are grown in seed flats in a green house condition for a period of 5 to 6 weeks. Then seedlings are cut at ground level with a razor blade and they are dried at 100° C for twenty-four hours and weighed. Seed lots with higher seedling dry weights are considered to have greater vigour than seed lots with low seedling dry weight. He also added that minimum two or three replications are required, with perhaps one hundred seeds planted per replication. If seed germination is low, determinations on a weight per seedling basis may be more meaningful man on per hundred seed basis. Gaffar *et at.* (1988) gave the similar opinion for seedling dry weight.

They revealed that for this method seedling of five to six weeks are dried in the oven at $100 \pm 5^{\circ}$ C for twenty-four hours.

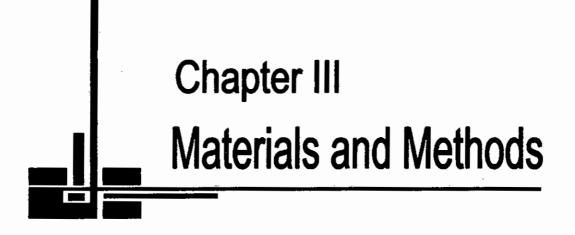
Hampton and Tekrony (1995) reported the seedling growth rate as a method of vigour test. In this method linear measurement of plumule growth was first suggested as a vigour test by Gern (1949), and has been successfully used on maize (Woodstock 1969) barley and wheat (Perry 1977). In this method the mean length of scedling is calculated after a specified time period for germination. In comparison of seed lots of the same cultivar of a species, seed

lots with greater mean seedling length are considered to have greater seed vigour than seed lots with smaller mean seedling length.

Gaffar *et al.* (1988) stated that seedling length measurement method is an easy method for vigour test. In this method firstly seeds are placed for germination. After an appropriate period of germination the total length of root and shoots are measured with a ruler. The seed lots with greater seedling lengths are considered to have greater seed vigour than seed lots with smaller seedling length.

Agrawal (1996) stated that in seedling length measurement method seeds are planted in a single row in a desirable medium. The tests are placed in germinators at a 45° angle. After an appropriate period of germination the length of roots or shoots is measured with a ruler. The average length of seedling per sample is calculated. Six replications of 15-25 seeds each are desirable.

Gurjar and Singh (2003) observed the effects of toxic metabolites from *Curvularia lunata* [*Cochliobolus lunatus*], *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. moniliforme* [*Gibberella moniliformis*] and *F. pallidoroseum* on the seed germination and seedling vigour of okra were determined. All treatments resulted in reduced seed germination and seedling vigour in terms of plumule and radicle length, with *F. oxysporum* treatment resulting in the highest reduction in the parameters measured.



CHAPTER III

MATERIALS AND METHODS

3.1. Experimental site

The seed quality analysis and the laboratory experiments were conducted in the Seed Pathology Lab in the department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207.

3.2. Experimental period

The experiments were conducted from July 2009 to June 2010.

3.3. Collections of seed samples

A total 50 samples of okra seeds were collected from nine districts of Bangladesh namely Bogra, Comilla, Chittagong, Dinajpur, Rangpur, Thakurgaon, Jessore, Norshingdi , Dhaka and other from research station' (BRAC, BARI, BADC, Mollic's and Lal Teer) during 2009 crop season. Of them 28 samples were obtained from 28 okra growers and 17 from the local market of the 9 districts and 5 samples from 5 research stations in Dhaka namely; BARI, BADC, BRAC, Mollic's and Lal Teer. The samples belonged to 3 varieties viz. Local-1 (22 samples), Local-2 (8 samples) and BARI Dherosh-1 (20 samples). The details of seed samples are given in Table 1. The samples were kept in polyethylene bags and preserved in a refrigerator at 4°C. Quality attributes and health status of the seed samples were determined by taking sub-samples from each of the samples. For quality tests and health status studies, working samples were drawn from individual sub-samples at the time of each test.

Table 1. List of seed samples used in experiment

Accession no.	Location of collection	Seed source	Variety
RS-1	Research station, Dhaka	BARI	BARI Dherosh-1
RS-2	Research station, Dhaka	BADC	BARI Dherosh-1
RS-3	Research station, Dhaka	BRAC	BARI Dherosh-1
RS-4	Research station, Dhaka	Mollic's seed	BARI Dherosh-1
RS-5	Research station, Dhaka	Lal teer	BARI Dherosh-1
BOG-1	Bogra	Retailer	BARI Dherosh-1
BOG-2	Bogra	Retailer	Local-2
BOG-3	Bogra	Farmer	BARI Dherosh-1
BOG-4	Bogra	Farmer	Local-1
BOG-5	Bogra	Farmer	Local-1
COM-1	Comilla	Farmer	Local-1
COM-2	Comilla	Farmer	Local-1
COM-3	Comilla	Retailer	BARI Dherosh-1
COM-4	Comilla	Farmer	Local-1
COM-5	Comilla	Retailer	Local-2
CHT-1	Chittagong	Farmer	Local-1
CHT-2	Chittagong	Farmer	Local-1
CHT-3	Chittagong	Retailer	BARI Dherosh-1
CHT-4	Chittagong	Farmer	BARI Dherosh-1
CHT-5	Chittagong	Farmer	Local-1
DIN-1	Dinajpur	Farmer	Local-1
DIN-2	Dinajpur	Retailer	BARI Dherosh-1
DIN-3	Dinajpur	Retailer	Local-2
DIN-4	Dinajpur	Farmer	Local-1
DIN-5	Dinajpur	Farmer	BARI Dherosh-1
RNG-1	Rangpur	Retailer	BARI Dherosh-1
RNG-2	Rangpur	Farmer	Local-1
RNG-3	Rangpur	Farmer	BARI Dherosh-1
RNG-4	Rangpur	Retailer	Local-2
RNG-5	Rangpur	Farmer	Local-1
THA-1	Thakurgaon	Retailer	BARI Dherosh-1
THA-2	Thakurgaon	Farmer	Local-1
THA-3	Thakurgaon	Farmer	Local-1
THA-4	Thakurgaon	Retailer	Local-2
THA-5	Thakurgaon	Farmer	Local-1
JES-1	Jessore	Farmer	Local-1
JES-2	Jessore	Farmer	Local-1
JES-3	Jessore	Retailer	Local-2
JES-4	Jessore	Retailer	BARI Dherosh-1
JES-5	Jessore	Farmer	BARI Dherosh-1
NOR-1	Norshingdi	Farmer	Local-1
NOR-2	Norshingdi	Retailer	BARI Dherosh-1
NOR-3	Norshingdi	Retailer	Local-2
NOR-4	Norshingdi	Farmer	Local-1
NOR-5	Norshingdi	Farmer	Local-1
DHK-1	Dhaka	Farmer	BARI Dherosh-1
DHK-2	Dhaka	Retailer	BARI Dherosh-1
DHK-3	Dhaka	Farmer	Local-1
DHK-4	Dhaka	Retailer	Local-2
DHK-5	Dhaka	Farmer	Local-1

3.4. Quality Tests

Seed quality in terms of moisture content, 1000-seed weight, purity, germination capacity and seedling vigour were determined (ISTA, 2006).

3.4.1. Moisture content

Moisture content of the seed samples was determined prior to temporary storage by a digital electric moisture meter and the results were expressed in percentage on wet weight basis (ISTA, 2006).

3.4.2. Thousand-seed weight

Weights of 1000-seeds were determined from sub-samples drawn from each seed samples. A total 3000 pure seeds, free from other seeds and inert matter were sorted out. They are divided into 3 working samples. Weights of 3 working samples were computed and recorded as 1000-seed weight (Ariyaratne, 1998).

3.4.3. Purity test

At least 140g of working samples were drawn from each seed samples of okra seed. They were sorted as pure seed, other seed and inert matter. Weight of each component was determined using electric balance. Percentage (w/w) of each component was determined, based on total weight of pure seed, other seed and inert matter (ISTA, 2006).

3.4.4. Germination test

Four hundred pure seeds (100 seeds / replication) were randomly selected from each sample. The selected seeds were sown in paper towel method at 50 seeds / paper towel. Seven days after sowing number of seedling emerged in each paper towel were recorded. Number of seedling emerged from 400 seeds were determined. The germination capacity was expressed in percentage based on total seed used in the test (ISTA, 2006).

The data were expressed in percentage based on total number of seeds plated. The germination was expressed in percentage which was calculated using following formula:

% Germination =
$$\frac{X_1}{X} \times 100$$
,

Where,

X = Total number of seeds per paper towel $X_1 =$ Number of seedlings per paper towel

3.4.5. Seedling vigor

For seedling vigor test, after 7 days of emergence 10 seedlings were randomly selected from each 100 seeds used for germination test. Altogether 30 seedlings were selected from each sub-sample. Root length (cm) and shoot length (cm) of the seedlings were recorded and mean values of the two parameters were computed. Vigour index was computed following a standard formula as suggested by Abdul-Baki and Anderson (1973), where,

Vigour index = [Mean root length (cm) + Mean shoot length (cm)] x Seed

Germination (%)



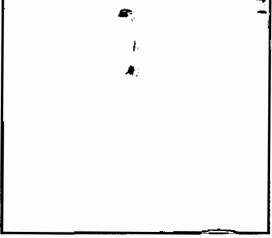


Plate1. Seedlings germinated in paper towel method

Plate 2. Healthy seedlings selected for measuring root and Shoot length (in cm).

3.5. Seed health test

Seed health in terms of fungi associated with okra seeds were tested following International Rules for Seed Health Testing using Blotter method (Anon. 1976).

3.5.1. Prevalence of fungi associated with randomly selected okra seeds

A sub-sample of 200 seeds was randomly selected from each sample. Seeds were plated on sterilized and moist filter paper in 9 cm petridish. Twenty five seeds were plated in each petridish maintaining equal distances from seed to seed. Before planting, the filter paper was autoclaved at 121° C temperature and 1 kg/cm^2 pressure for 20 minutes. After plating the seeds were incubated at $25\pm$ 4° C temperature. To keep the filter paper moist, sterilized water was added whenever necessary.

Data on germination and prevalence of seed-borne fungi grew on the plated seeds were recorded after seven days of incubation. Fungi associated with the seeds were observed under a binocular stereo dissecting microscope. Based on the morphological characters fungi were identified using appropriate keys (Barnett 1967, Robert and Streets 1982, Booth 1971, Ellis 1971, Mathur and Kongsdal 2003). When the identification of fungus was not possible by observing the growth characteristics under stereo-microscope, temporary mounts were prepared and examined under a compound microscope for detail morphology. Germination and seeds yielding different fungi were expressed in percentage based on total seeds plated.

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3.5.2. Prevalence of fungi associated with okra seeds under different grades

Seeds of ten selected samples, one from each district, were categorized in four grades (Fig. 1) based on their physical conditions observed under a hand lens. A total of 400 seeds were taken randomly from each of the six selected samples.

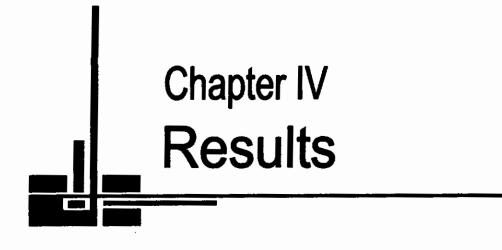
The grades were as follows:

- Grade-1 (G-I) : Apparently healthy seeds
- Grade-2 (G-II) : Discolored seeds
- Grade-3 (G-III) : Spotted seeds
- Grade-4 (G-IV) : Shriveled and broken seeds

Seeds under each grade were plated on moist sterilized filter paper following the procedures as described earlier and incubated at 25±4° C temperature for 7 days. Data on germination and prevalence of seed-borne fungi grew from plated seeds were recorded. Germination and seeds yielding different fungi were expressed in percentage based on total number of seeds plated.

3.6. Analysis of data

The data of all tests were analyzed statistically for analysis of variance (ANOVA) using MSTAT-C computer program. The means were compared following Least Significant Difference (LSD) at 1% level of significance using same computer program. Whenever necessary the data were transformed before statistical analysis following appropriate method.



CHAPTER IV RESULTS

4.1. Moisture Content

The average moisture content of collected okra seed obtained from 10 different locations of Bangladesh varied significantly from 13.31 to 11.12% (Figure 1). The maximum moisture content (13.31%) was found in seeds collected from Chittagong, followed by Rangpur (12.78%). The minimum moisture content (11.12%) was recorded at research' station, Dhaka followed by Thakurgaon (11.16%).

Moisture content of seed varied from variety to variety. The lowest moisture content was found in variety BARI Dherosh-1 (11.41%) and highest (12.16%) was in Local-1 variety (Table 3).

Variations of moisture content were also observed among the seed samples collected from research station' seed, retailer' seed and farmer' saved seed which was 11.12%, 11.55% and 12.05% respectively (Table 4).



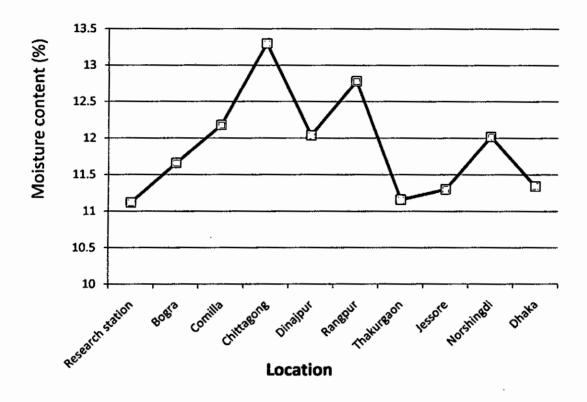


Figure 1. Moisture content (%) of okra seed collected from 10 different locations of Bangladesh.

4.2. Thousand Seed weight

In 50 seed samples collected from 10 different locations, 1000-seed weight varied significantly from 54.18g to 69.11g. The highest 1000-seed weight (69.11 g) was in the samples collected from research station' followed by Chittagong and Thakurgaon (62.02 g and 61.95 g, respectively) (Table 2). The lowest 1000-seed weight (54.18 g) was in the samples collected from Jessore which is statistically similar with Norshingdi (54.40 g).

Similarly, varietal differences in terms of 1000-seed weight were also differed significantly. Significantly highest 1000-seed weight (60.59 g) was observed in BARI Dherosh-1 and 1000-seed weight of Local-1 and Local-2 was 57.37 g and 55.85 g, respectively (Table 3).

The research station' seeds, retailer' seeds and farmer' saved seed also showed variation in 1000-seed weight. The maximum weight (69.11 g) was found in research station' seed and the minimum (57.44g) were found from retailer' seeds (Table 4).

Location	1000 seed	Purity	Germination	Mean Root	Mean shoot	Vigour Index
	weight (g)	(%)	(%)	Length (cm)	Length (cm)	
Research station'	69.11 a	96.94 a	81.93 a	9.66 a	9.39 a	1585.0 a
Bogra	58.72 c	95.10 d	57.07 e	8.71 bc	7.86 c	1017.0 d
Comilla	57.30 e	96.18 b	49.27 f	7.39 e	6.45 e	689.7 f
Chitagong	62.02 b	96.84 a	59.53 d	8.49 d	7.35 d	996.6 d
Dinajpur	57.89 d	93.68 e	46.60 g	6.97 f	6.35 ef	626.1 g
Rangpur	55.74 g	93.89 e	24.60 j	5.74 h	5.29 h	273.7 i
Thakurgaon	61.95 b	94.06 e	34.47 i	7.00 f	6.24 fg	736.0 e
Jessore	54.18 h	95.83 bc	61.53 c	8.89 b	7.93 с	1102.0 c
Norshingdi	54.40 h	95.65 c	36.40 h	6.14 g	6.07 g	458.5 h
Dhaka	56.66 f	95.79 bc	65.27 b	8.64 cd	8.74 b	1169.0 b
LSD (p≥0.01)	0.35	0.41	2.40	0.21	0.20	32.45
CV (%)	0.25	0.18	1.90	1.14	1.14	1.16

Variety	Moisture Content (%)	1000 seed weight (g)	Purity (%)	Germination (%)	Mean Root Length (cm)	Mean shoot Length (cm)	Vigour Index
Local 1	12.16 a	57.37 b	94.81 c	41.49 c	6.95 c	6.34 c	582.8 c
Local 2	11. 72 b	55.84 c	95.73 b	55.75 b	7.58 b	6.98 b	856.2 b
BARI dherosh 1	11.41 c	60.59 a	96.76 a	66.75 a	8.84 a	8.23 a	1204.0 a
LSD _(p≥0.01)	0.24	0.17	0.12	1.72	0.21	0.17	42.07
CV (%)	0.51	0.08	0.03	0.84	0.74	0.69	1.27

Table 3. Quality of okra seeds obtained from three okra varieties

Table 4. Quality of research station' seeds, retailer' seeds and farmer' saved seeds

Source	Moisture Content (%)	1000 seed weight (g)	Purity (%)	Germination (%)	Mean Root Length (cm)	Mean shoot Length (cm)	Vigour Index
Research station	11.12 c	69.11 a	96.94 a	81.93 a	9.66 a	9.39 a	1585.0 a
Retailer' seed	11.55 b	57.44 c	95.82 b	57.82 b	7.89 b	7.24 b	921.0 b
Farmer' saved seed	12.05 a	57.78 b	92.86 c	46.10 c	7.35 c	6.73 c	703.0 c
LSD _(p≥0.01)	0.21	0.29	0.31	2.61	0.24	0.21	38.62
CV (%)	0.47	0.12	0.09	1.12	0.80	0.67	0.96

4.3. Purity

The purity percentage of seeds varied with location, variety and source of seed collection. In case of location highest purity percentage was found in Chittagong (96.84%) which was similar with research station' seeds (96.94%). The lowest purity percentage was found in Dinajpur (93.68%) which was at par with Rangpur (93.89%) and Thakurgaon (94.06%) (Table 2).

Varietal difference also showed significant difference in purity percentage. The highest purity percentage (96.76%) was obtained from BARI Dherosh-1 and the lowest purity percentage (94.81%) was found from Local-1 variety (Table 3).

There was also significant difference in purity percentage of research station' seeds, retailer' seeds and farmer' saved seeds. Purity percentage of research station' seeds, retailer' seeds and farmer' seeds was found 96.96%, 95.82% and 92.86%, respectively. Most of the samples from farmer' saved seed had lower purity percentage than the research station' seeds and retailer' seeds (Table 4).

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4.4. Germination capacity

Germination percentage of 50 okra seed samples significantly varied with locality of sample collection, source and variety of collected sample. The germination percentage of okra seeds in 10 different location ranges from 24.61% to 81.93%. Highest germination percentage (81.93%) was obtained from Research station' seeds followed by Dhaka (65.27%) (Table 2). The lowest germination percentage (24.60%) was recorded from seeds collected from Rangpur followed by Norshingdi (36.40%).

Again seed germination percentage also varied in terms of varietal variation. The significant higher germination percentage (66.75%) was obtained from BARI Dherosh-1 followed by local-2 variety (55.75%) (Table 3). The lowest germination percentage (41.49%) was recorded from variety local-1.

In case of source of the seed the highest germination percentage (81.93%) was recorded from research station' seeds followed by retailer' seeds (57.82%) where the lowest germination percentage (46.10%) was obtained from farmer' saved seed (Table 4).

4.5. Seedling Vigour

Seedling vigour differs significantly in terms of locations, variety and source of the seed collection. In seedlings, root length ranged from 5.74 cm to 9.66 cm, shoot length from 5.29 cm to 9.39 cm and vigour index from 273.7 to 1585.0 in 10 different locations. The longer root and shoot length (9.66 cm and 9.39 cm respectively) obtained from research station' seeds and shorter root length (5.74 cm and 5.29 cm, respectively) from Rangpur. The higher vigour index (273.71) from Rangpur. The higher value of vigour index refers to the high vigour of seeds. Increase in seedling vigour of seed samples occur mostly because of increase in 1000-seed weight.

BARI Dherosh-1 produce significantly longer root and shoot length (8.84 cm and 8.23 cm, respectively) and higher vigour index (1204.0) among the three varieties and shorter root and shoot length (6.95cm and 6.34 cm, respectively) and minimum vigour index (582.8) from local-1 variety (Table 3).

From table 4 it is evident that research station' seeds showed significantly longer root and shoot length (9.66 cm and 9.39 cm, respectively) and higher vigour index (1585.0) than the retailer' seeds (7.89 cm, 7.24cm and 921.0, respectively) and farmer' saved seeds (7.35cm, 6.73cm and 703.0). Present study showed that farmer' saved seeds less vigorous than the research station' seeds and retailer' seeds and retailer' seeds gave intermediate values.

4.6. Prevalence of seed-borne fungi

Altogether seven fungal species belong to six genera were found to be associated with the seed samples from 10 different locations. The six genera were Fusarium, Aspergillus, Macrophomina, Colletotrichum, Rhizopus and Curvularia.

The percentages of total seed-borne infection of various fungi in different location range from 56.32% to 95.96%. The highest total fungal prevalence (95.96%) was recorded in the Rangpur district and lowest fungal prevalence (56.32%) from research station' seeds (Figure 2).

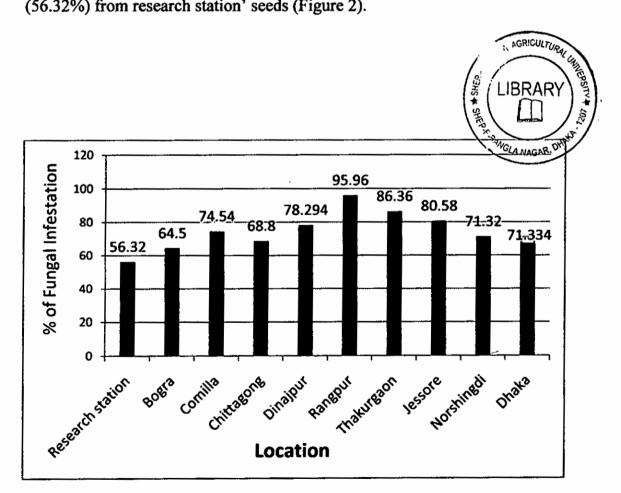


Figure 2. Percentages of total seed-borne fungal infection in okra seeds collected from different locations of Bangladesh.

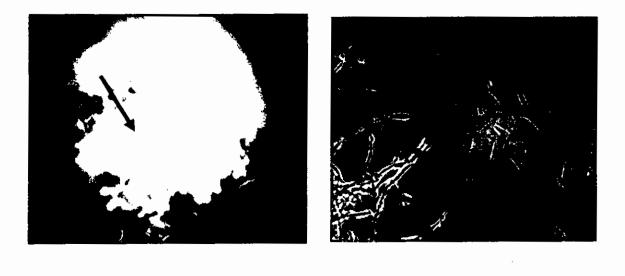
The identified seven fungal species that encountered were Fusarium spp., Aspergillus niger, Aspergillus flavus, Macrophomina phaseolina, Colletotrichum dematium, Rhizopus spp. and Curvularia spp (Plate 3-8). Among these fungi Aspergillus spp. (A. niger and A. flavus) were found to be the most prevalent, which was followed by the species of Fusarium spp.

* * ****

The most predominant fungi *Aspergillus flavus* was present in all the seed samples and its prevalence varied from 21.0% to 30.13% (Table 5). The highest infection (30.13%) was found in Rangpur and lowest (21.0%) in Dinajpur. The highest infection of *Aspergillus niger* was recorded 20.20% and lowest from Dhaka (15.80%). *Fusarium* spp also found in all samples. The highest infection of *Fusarium spp* was recorded 27.73% in Thakurgaon which is at par with Norshingdi (27.27%) and the lowest (19.47%) in Bogra which is statistically similar with Norshingdi (20.27%). The prevalence of other fungi viz. *Macrophomina phaseolina, Colletotrichum dematium, Rhizopus* spp. and *Curvularia* spp varied from 1.20% to 00%, 16.20% to 3.20%, 3.38% to 1.07% and 3.47% to 0.40%, respectively. These fungi were also reported to be seedborn in okra by Fakir (1980), Gupta *et al.* (1989) and Fernandes *et al.* (1992).



Plate 3. Pycnidia formed by *Macrophomina phaseolina* on root tip (A) & abundant conidia released from pycnidium (B).



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Plate 4. Fusarium solani infected okra seed (A), microscopic view of conidiophores & conidia (B).

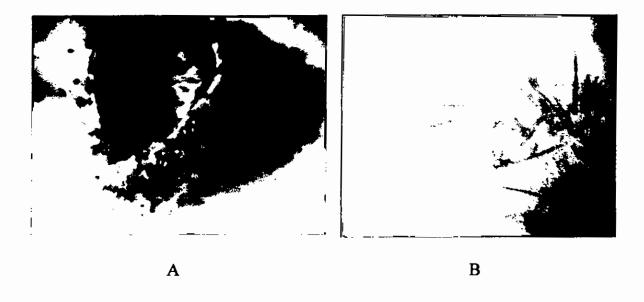


Plate 5. Colletotrichum infected okra seed (A), Acervuli of Colletotrichum spp. under compound microscope (B).

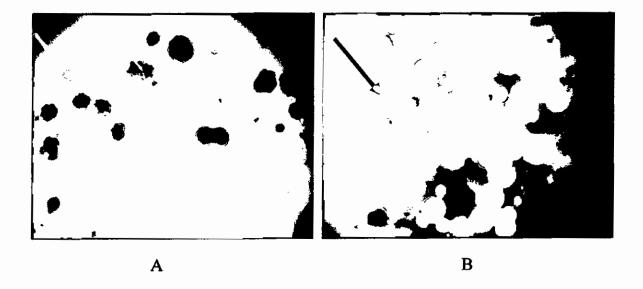


Plate 6. Aspergillus niger (A) and Aspergillus flavus (B) infected okra seed.



Plate 7. Conidia, conidiophores & mycelia of *Curvularia spp.* isolated from okra seed.

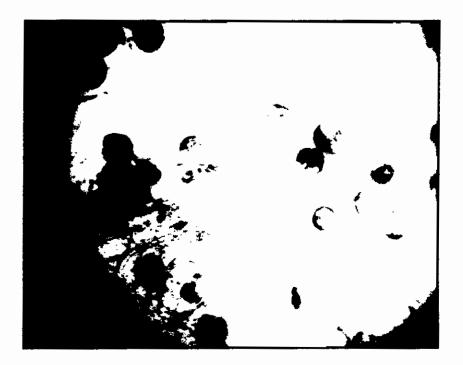


Plate 8. Sporangia of *Rhizopus* spp. isolated from okra seed.

Location	Fusarium spp.	Aspergillus niger	Aspergillus flavus	Macrophomina phaseolina	Colletotrichum dematium	Rhizopus spp.	Curvularia spp.
Research station	17.27 a	15.53 a	15.40 a	0.00 a	3.20 a ·	1.40 a	0.40 a
Bogra	19.47 b	20.07 d	17.20 a	0.53 bc	3.60 ab	1.40 a	2.40 cd
Comilla	24.27 c	19.80 d	16.40 a	1.00 d	7.40 d	3.33 b	2.53 d
Chitagong	17.47 a	18.73 bcd	24.53 de	1.07 d	4.67 c	1.33 a	1.13 b
Dinajpur	27.27 d	19.33 cd	21.00 Ъ	0.43 b	3.87 b	3.13 b	3.47 c
Rangpur	25.20 c	20.20 d	30.13 f	0.27 ab	15.13c	3.38 b	2.33 cd
Thakurgaon	27.73 d	16.40 abc	21.60 bc	0.87 cd	16.20 f	1.13 a	3.40 c
Jessore	25.27 c	19.53 d	25.27 e	1.20 d	5.13 c	1.53 a	2.87 cd
Norshingdi	20.27 b	18.07 abcd	23.07 cd	0.00 a	7.13 d	1.07 a	1.87 c
Dhaka	24.33 c	15.80 ab	23.07 cd	0.33 ab	6.93 d	1.13 a	2.60 d
LSD (p≥0.01)	1.01	2.99	1.84	0.38	0.60	0.73	0.60
CV (%)	1.88	6.94	3.60	28.47	3.47	16.44	11.19

Table 5. Incidence of different fungi associated with okra seeds collected from different locations

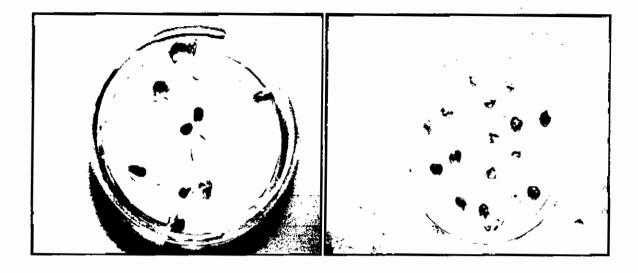


Plate 9. Germination of healthy okra seed

Plate 10. Pathogen infected okra seed with lower germination.

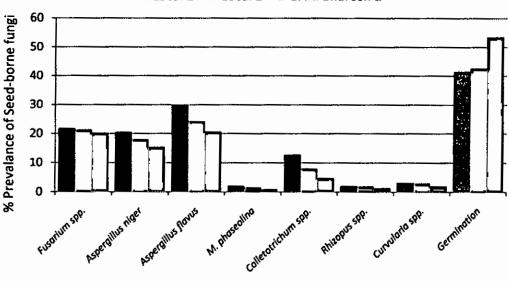


Plate 11. Fusarium spp. infected seed Plate 12. M. phaseolina infected okra in petridish.

seed in petridish

In blotter test, the germination percentage of the seeds was lower where the prevalence of fungi higher (Plate 9-12). Though exceptions were observed in case of some seed samples, it revealed that seed-born fungi affected seed germination. It was reported by other workers (Khanzada *et al.*, 1988, Prasad *et al.*, 2000).

Infection of all fungi was higher in local-1 variety followed by local-2. The lowest infection of fungi was observed in BARI Dherosh-1. Infection of *Fusarium* spp., *Aspergillus niger*, *Aspergillus flavus*, *Macrophomina phaseolina*, *Colletotrichum dematium*, *Rhizopus* spp. and *Curvularia* spp. in local-1 variety was 21.82%, 20.51%, 29.90%, 2.1%, 12.76%, 2.07% and 3.15%, respectively (Figure 3).



Local 1 Local 2 BARI dharosh 1

Figure 3. Percent prevalence of seed-borne fungi in three okra varieties.

Marked variation in the prevalence of fungi among the research station' seeds, retailer' seeds and farmer' saved seeds. *Aspergillus niger* was 20.42% in farmer' saved seeds, 15.51% in retailer' seeds and 15.52% in research station' seeds. *Aspergillus flavus* was 19.68% in farmer' saved seeds, 26.98% in retailer' seeds and 15.38% in research station' seeds. Infection of *Fusarium* spp was 23.58% in farmer' saved seeds, 22.18% in retailer' seeds and 17.2% in research station' seeds (Figure 4). Results indicated that decreased seed germination in farmer' saved seed was due to low quality seeds with high incidence of seed-born fungi.

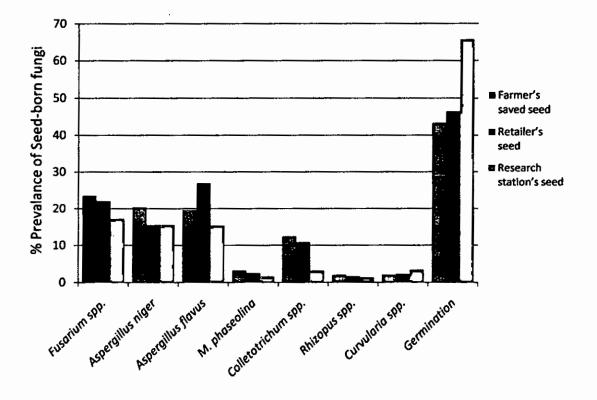


Figure 4. Percent prevalence of seed-borne fungi in farmer', retailer' and research station' seeds.

4.7. Prevalence of seed-borne fungi and germination of different grades of seeds

In 50 seed samples the percentage of four different grades of seed varied. The apparently healthy seeds (G–I), discolored seeds (G-II), spotted seeds (G-III) and shriveled & broken seeds (G–IV) were found 90.83%, 3.59%, 2.40% and 3.18%, respectively (Figure 5).

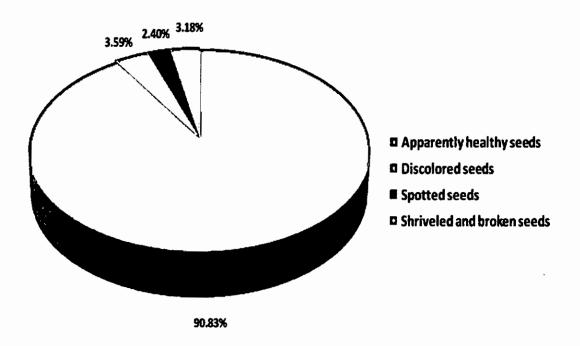


Figure 5. Percentage of different graded seeds of okra collected from different locations.



The percentage of four different grades of seeds in ten seed samples collected from different locations is presented in Table 6. Percentage of apparently healthy seeds grouped as Grade-I ranged from 86.50-96.03% in ten selected samples. In the same samples it was much higher in all the ten seed samples. The percentage of seeds under Grade-II, Grade-III and Grade-IV ranged from 2.43-5.55%, 0.81-4.22%, and 0.25-5.13%, respectively.

Samples	Grade					
	Grade -I	Grade -II	Grade -III	Grade –IV		
RS -2	96.03 a	2.43 a	1.28 b	0.25 a		
BOG-1	86.50 j	4.93 c	4.22 f	4.35 e		
COM-4	92.22 e	2.85 b	3.22 e	1.72 b		
CHT-3	94.18 c	2.50 bc	1.56 b	1. 76 b		
DIN-2	89.72 h	5.10 c	2.63 d	2.55 c		
RNG-5	87.63 i	5.55 d	3.30 e	3.52 d		
THK-3	90.20 g	2.73 bc	1.94 c	5.13 f		
JES-4	95.00 b	2.60 bc	0.81 a	1.59 b		
NOR-2	93.00 d	2.50 bc	1.27 b	3.23 d		
DHK-1	90.65 f	4.87 c	2.00 c	2.48 c		
LSD _(p≥0.01)	0.40	0.39	0.33	0.29		
CV (%)	0.19	4.60	6.41	4.54		

Table 6.	Percentages of different graded seeds of okra in 10 selected
	samples

- Grade -I : Apparently healthy seeds
- Grade -II : Discolored seeds
- Grade -III : Spotted seeds
- Grade -IV : Shriveled and broken seeds

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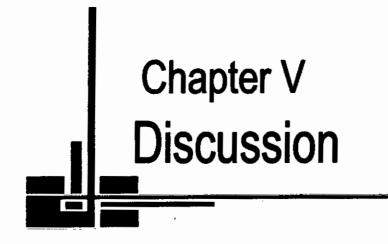
Among the fungal genera recorded from seeds under different grades *Aspergillus, Fusarium* and *Macrophomina* were predominant. The prevalence of all the fungi was significantly lower in Grade –I seeds as compared to other grades. On the other hand, seeds under Grade –IV yielded significantly the highest populations of three predominant fungal genera. The percentage of seeds yielding the three predominant genera ranged from 12.60-65.43%, 8.43-29.20% and 0.63-3.20%, respectively under all grades having the lower value in Grade-I and the higher value in Grade-IV (Table 7). Germination percentage was 70.43, 41.67, 39.73 and 16.57 under Grade-I, Grade-II, Grade-III and Grade-IV, respectively. The differences in germination under different grades of seeds were significant (Table 7).

Samples	Aspergillus	· Fusarium spp.	Macrophomina	Germination
	spp.		phaseolina	
Grade -I	12.60 a	8.43 a	0.63 a	70.43 a
Grade -II	33.57 b	20.40 b	1.63 b	41.67 b
Grade –III	43.70 c	24.47 c	2.10 c	39.73 c
Grade -IV	65.43 d	29.20 d	3.20 d	16.57 d
LSD _(p≥0.01)	0.87	1.10	0.27	1.53
CV (%)	0.74	1.77	4.66	1.20

Table 7. Percentages of seed-borne fungi and seed germination in different graded seeds of okra in 10 selected samples

- Grade –I : Apparently healthy seeds
- Grade -II : Discolored seeds
- Grade -- III : Spotted seeds
- Grade –IV : Shriveled and broken seeds





CHAPTER V DISCUSSION

The present study was undertaken to assess the status of seed health and quality of farmer' saved okra seeds in Bangladesh with the ultimate objectives for its improvement. Quality and health status of okra seed collected from research stations, retailers and other from farmers were determined. Total fifty seed samples were collected from nine districts of Bangladesh. Of them 28, samples were obtained from farmers and 17 from the retailers and 5 samples from research stations.

The seed quality analysis revealed that the average moisture content of the research station' seeds, retailer' seeds and farmer' saved okra seeds varied from 11.12 to 12.05%. This was probably due to the differences in the degree of drying of seeds. Again, this difference could be due to the ignorance of the farmers about the deleterious effect of high moisture content in seeds. Or, it could be due to the exposure of seeds to wet weather when okra crop is harvested and processed. The national standard for moisture content of okra seed is 10% (Anon, 2006). But, moisture content data obtained in the present study showed that all the hundred seed samples had higher moisture content than that of the national standard. High moisture content in seeds stored with higher moisture content is vulnerable to the attack of storage fungi and insects,

which often cause considerable losses through reduction in germination (Christensen and Kaufmann, 1963; Islam *et al.* 1997).

The 1000-seed weight of okra varies from 69.11 g, 57.44 g and 57.78 g among the research station' seeds, retailer' seeds and farmer' saved seeds. Low 1000seed weight was found in farmer' saved seeds and retailer' seeds as compared to research station' seeds. Pandita and Randhawa (1992) reported that with increase in seed size, the field emergence is also increased.

The purity of research station' seeds, retailer' seeds and farmer' saved seeds was 96.94%, 95.82%, 92.86 respectively. It is general principles that germination test should be performed using only pure seeds. So, germination capacity of seed sample is dependent on purity percentage of seed. Seed contamination *viz.* weed seed, other seed, inert matter found in most of the samples collected from all the 10 locations. These contaminants occurred in trace to appreciable extent depending on the type of contaminants and sources of seed collections. This situation indicates that farmer' saved okra seed is not of good quality. Occurrence of percentage of seeds of other crop varieties in farmer' saved okra seeds in the present study indicates that farmers are not much careful about the varietal mixture in their saved seeds. Presence of seeds of other okra varieties as well as seeds of other crops in a given seed lot may affect the health of the seed lot through mixture of seeds of susceptible varieties/crops with the saved seeds of a resistant variety.

The germination percentage of research station' seeds, retailer' seeds and farmer' saved seeds was 81.93%, 57.82% and 46.10% respectively. The results reflected that farmer' saved seeds showed lowest germination capacity. Variation in germination among the seed samples may be due to high moisture content, low1000-seed weight and low purity in farmer' seeds as compared to retailer' seeds and research station' seeds. Varies and Agrawal (1992) also observed that seed germination decreased with low purity, high temperature and high moisture prevalence. Present study showed that farmer' saved seeds (703.0) were less vigorous than the research station' seeds (1585.0) and retailer' seeds (921.0) gave intermediate values. Matthews (1980) reported that field emergence of seed lots with high germination capacity have been attributed to differences in seed vigour. Present investigations revealed that higher seed weight and seed samples with high germination percentage increased seedling growth in terms of root and shoot length. Similar results were reported by Pandita and Randhawa (1992). There are many factors which affect seed vigour. Among them genetic constitution, environment and nutrition of mother plant, stage of maturity at harvest time (Shepard et al., 1995), size and weight (Shepard et al., 1996), mechanical integrity, seed ageing, pathogens and storage environment are most important.

Marked variation in the prevalence of fungi were found among the research station' seeds, retailer' seeds and farmer' saved seeds. Higher percentages of fungal infection were found in the farmer' saved seeds. Because, presence of inert matter, weed species, other seeds in farmer' saved seeds may carry

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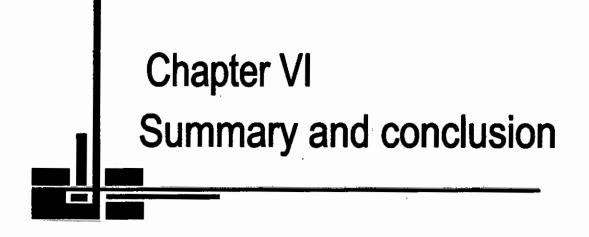
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inoculants of fungal pathogen like Fusarium spp., Aspergillus spp., Rhizopus spp. Thus the presence of inert matter in farmer' saved seeds posses risk as regard to contamination of seed lot by the propagules of pathogenic fungi. Therefore, studies need to be undertaken on the role of inert matter present in farmer' saved okra seeds in carrying pathogenic fungi. Of the seven fungal pathogens, Fusarium spp. and Aspergillus flavus were most predominant. The pathogen had very high incidence ranging from 27.730-19.47% and 21.0-30.13%, respectively. Presence of these fungi in the tested seed samples depict that the health status of okra seed samples were not satisfactory. This fungus may create alarming disease outbreaks in the fields resulting heavy yield losses to the crop as well as hampering in quality seed production. Occurrence of the storage fungus, A. flavus in the test seed samples also questions the keeping quality of farmers saved okra seeds in storage as the pathogen is known to cause germination loss in many crops. The above mentioned facts can be supported from the germination test record in the present study, where original farmers saved seeds had always poor germination, produced much lesser number of normal seedlings and resulted higher number of dead seeds, abnormal or diseased seedlings and low seedling vigour. On the contrary, best seeds obtained from research station' seeds. In the germination test in natural soil the research station' seeds result markedly higher percentage of normal seedlings, lesser percentage dead seeds, abnormal seedlings including diseased seedlings and high seedling vigour.

Seeds were graded in 4 grades viz. apparently healthy seeds (G-I), discolored seeds (G-II), spotted seeds (G-III), shriveled and broken seeds (G-IV). In 50 seed samples an average, 90.83% were apparently healthy seeds, 3.59% discolored seeds, 2.40% spotted seeds and 3.18% shriveled & broken seeds respectively. Percentages of abnormal seeds were higher. This is an alarming situation of okra seeds production in Bangladesh. The situation demands more careful attention to seed crop management and processing of seeds after harvest specially during cleaning operations. Results of the present investigation showed that apparently healthy seeds (Grade -I) had low seed-borne fungal infections and gave higher percentage of germination as compared to other seed grade, which yielded the highest incidence of seed-borne fungi and lowest seed germination. Rahman et al. (2002) reported that in rice seeds, percent incidence of fungal pathogens and inoculums load was higher in farmers own seeds compared to apparently healthy seeds obtained by manual sorting of farmers seeds. Malaker and Mian (2008) also reported that the prevalence of fungi was low and maximum of 99.0% germination was found in apparently healthy seeds of wheat.

In the present study, it was found that size and weight of okra seeds had profound effect on germination and seedling vigour. Shriveled and broken seeds (G-IV) seeds showed lower germination and seedling vigour compared to bigger and heavier seed (G-I). The results suggested that grading of seeds before sowing and use of apparently healthy seeds increase percentage of germination and decrease incidence of disease. So, it is recommended to sort out apparently healthy seeds from farmer' saved okra seeds before sowing.

From the foregoing discussion, it is clearly evident that the health status of farmer' saved seeds is poor. It is therefore, suggested to provide proper training to the farmer for better seed crop management and proper storage of okra seeds. This will help to improve the seed health and quality of okra seeds at farmer's level and contribute for higher yield per unit area.



CHAPTER VI SUMMARY AND CONCLUSION

An investigation was conducted in Seed Pathology Lab in the department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207 to determine the seed quality and seed health status of okra seeds collected from research stations, retailers and farmers saved seeds of nine different districts. A total 50 sample of okra seeds were collected, out of which 28 samples were farmers saved seeds, 17 from retailers and 5 samples from research stations.

Seed quality in terms of moisture contents, 1000-seed weight, purity, germination capacity and seedling vigour were determined by following standard method (ISTA 2006). Seed health in terms of fungi associated with okra seeds was tested following International Rules for Seed Health Testing using Blotter method. Seed grading was carried out based on their physical conditions observed under hand lens in four grades. The moisture content varied from 11.12% to 12.05% in different sources of seed collection, the minimum moisture content (11.12%) was recorded at research station' seeds and the maximum moisture content (12.05%) was found in the farmer' saved seeds. Moisture content of seed also varied from variety to variety. The research station' seeds, retailer' seeds and farmer' saved seeds also showed variation in 1000-seed weight. The maximum 1000-seed weight (69.11 g) was found in research station' seeds and the minimum 1000-seed weight (57.44g) were found from retailer' seeds. Varietal

differences in terms of 1000-seed weight were also differed significantly. Most of the samples had less than 98% purity. The purity percentage of seeds varied with locality and variety. The highest purity percentage (96.94%) was obtained from research station' seeds and lowest purity percentage (94.86%) was found in farmer' saved seeds. In case of sources of seeds collection, the highest germination percentage (81.93%) was recorded from research station' seeds and the lowest germination percentage (46.10%) were obtained from farmer' saved seeds. The results reflected that farmer' saved seed showed germination capacity far below the recommended seed standard. Seedling vigour differs significantly in terms of locations, variety and source of the seeds. Present study showed that farmer' saved seeds (703.0) was less vigorous than the research station' seeds (1585.0).

Standard Blotter methods were followed for the detection of seeds associated with fungi and 7 fungi were detected under six genera. They were *Fusarium spp., Aspergillus niger, Aspergillus flavus, Macrophomina phaseolina, Colletotrichum dematium, Rhizopus* spp. and *Curvularia* spp. The most predominant fungi was *Aspergillus flavus* (30.13%) followed by *Fusarium spp.* (27.71%), *Aspergillus niger* (20.20%) in terms of locations. They were also predominated in terms of variety and sources. Infections of all fungi were higher farmer' saved seeds and the lowest infection of fungi was observed in research station' seeds.

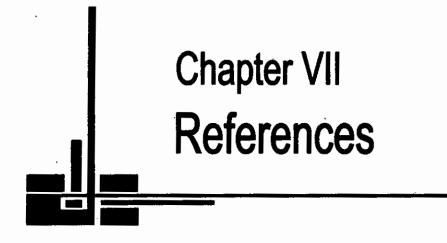
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The seeds samples that's graded as apparently healthy seeds (G-I), discolored seeds (G-II), spotted seeds (G-III) and shriveled & broken seeds (G-IV) were found 90.83%, 3.59%, 2.40% and 3.18%, respectively. Results of the present investigation showed that apparently healthy seeds (G-I) had low seed-borne fungal infections and gave higher percentage of germination (70.43%) as compared to seeds under Grade-IV, which yielded the highest incidence of seed-borne fungi and lowest seed germination (16.57%).

Based on findings of the present investigation, following conclusions were drawn-

- Seed samples of okra collected from farmers have high moisture content and low germination capacity, low purity percentage and less vigour in contrast with research station' seeds and retailer' seeds.
- Fusarium spp., Aspergillus niger, Aspergillus flavus, Macrophomina phaseolina, Colletotrichum dematium, Rhizopus spp. and Curvularia spp. are seed-borne fungi of okra seeds. Among them Aspergillus spp. was severe.
- Apparently healthy seeds under G-I yielded lower fungal incidence and increased germination percentage appreciably.







REFFERENCES

- Abdul- Baki, A. A. and Anderson, J. D. (1973). Vigour determination in soybean by multiple criteria. Crop Sci. 10:31-34.
- Abduhu, M. (2007). Quality of okra seed collected from farmers and control of seed borne pathogens by seed treatment. M.S thesis. Department of seed technology, Bangabandhu Sheikh Mujibur Rahman Agricultural University.
- Adisa, V.A. and Aborisade, A. T. (1987). Seed borne mycoflora of two okra cultivars and their effects on seed quality. *Fitopathologia Brasileria*.
 12(4):388-390.
- Adisa, V. A. and Aborisade, A. T. (1988). Seed-borne mycoflora of two okra cultivars and their effects on seed quality. *Rev. Plant Pathol.* 67(11): 618.
- Agrawal, R. L. (1996). Seed technology. 2nd Edn., Oxford & IBH Publishing Co. Pvt. Ltd. 66, Janpath, New Delhi. p. 829.
- Agrawal, S. and Singh, T. (2000). Effect of extra and intra embryonal infection of *Macrophomina phaseolina* on disease transmission in okra seeds. J. Mycology Plant Pathol. India, **30**(3): 355-358.

- Ahamad, S., Khan, A., Chauhan, S. S. (2001). Studies on seed-borne nature of Macrophomina phaseolina in okra. Annals of Plant Protection Sci., India. 9(1): 152-154.
- Akanda, A. M. (1993). Role of seed borne diseases of crops on crop production. Progress in Plant Pathology. Bangladesh Phytopathology Society. p. 34-43.
- Alam, A.K.M.A. (2001). Studies on the quality of vegetable seeds available in the market. M.S. thesis, Department of Horticulture, Bangladesh Agricultural University, Mymensingh. p. 90.
- Alam, M.M. 2002. Studies on the health of some vegetable seeds collected from different sources. M.S. Thesis. Dept. of plant Pathology, Bangladesh Agricultural University, Mymensingh.
- Alam, A. A. K. M. and Baziur Rashid, A. Q. M. (2005). Quality of okra seeds available in the Market. J. Agric. Rural Dev. 3 (1&2): 111-115.
- Anam, M. K. (2000). Effect of seed treatment on the incidence of seed borne fungal diseases and production of healthy seeds of okra. M.S. Thesis,
 Dept. of Plant Pathology, Bangladesh Agricultural University,
 Mymensingh, Bangladesh.
- Anonymous. (1976). International Rules for Seed Testing. International Seed Testing Association (ISTA). Seed Sci. and Tech. 24: 29-72.

- Anonymous, (1995). Winter Vegetable and Spices Production. Training Manual, Horticulture Research and Development Project (FAO/UNDP/AsDB Project, BGD/87/025) in collaboration with the Department of Agricultural Extension (DAB) and Bangladesh Agricultural Development Corporation (BADC). Dhaka. p. 284.
- Ariyaratne K. A. D. (1998). Seed Testing Manual. Seed Certification Agency. Gazipur, Bangladesh. 45-86.

Ashrafuzzaman, H. (1991). A Text Book of Plant Pathology. pp. 374-375.

- Atia, M. M., Tohamy, M. R. A., 2004. First record of Alternaria leaf spot disease on okra in Egypt. Egyptian J. Phytopathol. 2004; 32(1/2): 141-142.
- BARI (Bangladesh Agricultural Research Institute). (2010). Annual progress report of okra cultivation. Bangladesh.
- Barnett, H. L. (1967). Illustrated genera of Fungi Imperfecti. Burgess Pubi. Co. Minneapolis U.S.A. p. 223.
- Bazlur Rashid, A. Q. M., Barma, A.C., Shaikh, M. A. Q. (1983). Seed borne fungi mung bean and their pathogenicity. *Bangladesh J. Botany.* 12(2): 223-224.
- Begum (2005). Pathogenicity of Macrophomina phaseolina and Fusarium verticilloides in okra. Integrative-Biosciences. 9(1): 37-40.

- Bhattiprolu, S.L and Rahman, M.A. (2008). Field screening of okra entries against yellow vein mosaic virus disease. *Progressive Research*, India. 3(1): 85-86.
- Booth, C. (1971). The genus Fusarium. Common. W. Mycol. Inst. Kew. Surrey, England. p. 236.
- Chassiar, A. (1984). Genetic Resources of the genus Abelmoschus. Mediterranean Rep. Int. Board Plant Genetic Res. Rome. p. 61.
- Chavan, R. A., Dhoke, P. K., Bharose, A. A. and Jadhav, V. T. (2003). In vitro efficacy of fungicides in controlling *Fusarium oxysporum* f. sp. *carthami. J. Soils* and *Crops.* 13(1):98-100.
- Cheema, D.S. and Jhorar, P. (1954). Environmental conditions influencing the development of anthracnose disease of okra. *Indian Phytopathology*. 29(3): 145-148.
- Chidambaram, P.S. and Mathur. B.S. (1975). Production of pycnidia by Macrophomina phaseolina. Trans. Br. Mycol. Soc. 64:24-25.
- Christensen, C.I. and Kaufmann, H. H. (1963). Deterioration of grains fungi. Ann. Rev. Phytopathol. 10 (1): 39-42.
- Dhingra, A. D. and Sinclair, J. B. (1973). Variation among the isolates of Macrophomina phaseolina (R. bat aticola) from different regions. Phytopathology. 7(76):200-204.

- Diaz, C., Hossain, M., Bose, M. L., Mercea, S. and Mew, T.W. (1998). Seed quality and effect on rice yield: Findings from farmerss participatory experiment in Central Luzon, Philippines. *Philippines. J. Crop. Sci.* 23(2): 111-119.
- Ellis, M. B. (1971). Dematiaceous Hyphomycetes. CMI, Kew, Surrey, England. p. 608.
- Esuruso, O. F., Ogundiram, S. A., Chheda, H. R. and Fatokun, D. O. (1975). Seed borne fungi and some fungal disease of okra in Nigeria. *Plant Disease Reports*. 59(8):660-663.
- Fakir, G. A. (1976). Detection of seed borne fungi of okra, their role and control: A monograph by the Danish Government Institute of Seed Pathology, Copenhagen, Denmark. p. 22.
- Fakir, G. A., Thirumalachar, M. J., Mathur, S. B. and Neergaard, P. (1977). Seed borne infection of *Macrophomina phaseolina* and *Colletotrichum dematium* in okra (*Hibiscus esculentus*) in Bangladesh. *Bangladesh J. Agric. Sci.* 4: 75-79.
- Fakir, G. A. (1980). An annotated list of seed borne disease in Bangladesh. Bangladesh Agriculture Information Service, Dhaka.
- Fakir, G. A. (1982). An annotated list of seed borne disease in Bangladesh. Bangladesh Agricultural Information Service, Dhaka. p. 17.

- Fakir, G. A. and Mridha, A. U. (1985). Die- back caused by Collectotrichum dematium and Macrophomina phaseolina a new disease of lady's finger (Hibiscus esculentus L.). Bangladesh J. Plant Pathol. 1:25-28.
- Fakir, G. A. (1986). Annual Progress Report. Seed Pathology sub-project. Department of Plant Pathology, Bangladesh Agricultural University. Mymensingh, p. 17.
- Fakir, G. A. (1987). Annual Progress Report. Seed Pathology sub-project.Department of Plant Pathology, Bangladesh Agricultural University.Mymensingh. p. 20.
- Fakir, G. A. (2000). An annotated list of seed borne disease in Bangladesh. Seed Pathology Laboratory. Dept. of Plant Pathology, Bangladesh Agricultural University. Mymensingh. p. 41.
- Fakir, G. A. (2001). An annotated list of seed borne disease in Bangladesh. Seed Pathology Laboratory. Dept. of Plant Pathology, Bangladesh Agricultural University. Mymensingh. p. 41.
- Fernandes, M, Do, C. A., Almeida, O. C., Conha, R. and Robbs, C. F. (1992). Preliminary studies of health testing in okra seed from different municipalities of *Rio de Janerio state. Rev. Plant Pathol.* 72(10):796.
- Gaffar, M. A., Iqbal, T. M. T and Islam, M. S. (1988). Weed and Seed (in Bangla). 1st Edn., Published by T. M. Jubair Bin Iqbal, Sirajgong, Bangladesh. p. 95.

- Gangopadhyay, S. and Kapoor, S. (1977). Control of Fusarium wilt of okra with seed treatment. Indian J. Mycology and Plant Pathol. 30 (1): 112-113.
- Gupta, K., Sindhu, I. R. and Sagufla, N. (1989). Seed mycoflora of Abelmoschus esculentus. Survey and enumeration. Acta botanica. 17(2):200-206.
- Gupta, K., Sindhu, I. R. And Naaz, S. (1989). Seed mycoflora of Abelmoschus esculentus (L.) Moench; A survey and enumeration. Acta-Botanica-Inica. 17 (2): 200-206.
- Gupta, D. K. and Choudhury, K. C. B. (1995). Seed borne fungi of bhindi, brinjal and chilli grown in Sikkim, India J. Mycol. and Pl. Pathol. 25 (3): 282-283.
- Gurjar, K. L., Singh, S. D. (2003). Effect of toxic metabolites of pathogenic seed borne mycoflora of okra on seed germination and seedling vigour.
 Plant Disease Research Ludhiana, India. 18(2): 172-173.
- Goel, S. K. and Mehrotra, R. S. (1973). *Rhizoctonia* root rot and damping-off of okra and its control. *Acta Botanica Indica* 1:45-48.
- Goel, S. K. and Mebrotra, R. S. (1977). Root and collar rot of okra caused by R. bataticola and its control. Indian Phytopathol. 30:112-113.

- Hampton, J. G. and Tekrony, D.M. (1995). Handbook of Vigour Test Methods.
 3rd Edn., The International Seed Testing Association (ISTA), Switzerland. pp. 92-100.
- Haware, M. P. (1971). Fungal microflora of seeds of *Pisum sativum* L. and its control. *Mycopath. Mycol. Appi.* 43:343-345.
- Hema, L. R., Srivanna, M. B., Kumar, V., Krishnappa, M., Prokash, H, S., and Shetty, H. S. (1989). Control of seed-borne fungi in okra by fungicidal seed treatment. *Indian J. Agril. Sci.* 61(10):778-782.
- Henz, G. P., Lopes, C. A., Reis, A. (2007). A novel postharvest rot of okra pods caused by *Rhizoctonia solani* in Brazil. *Fitopatologia Brasileira*. 32(3): 237-240.
- Huan, K. I. and Jamil, H. U. (1983). Studies on the efficiency of seed testing fungicides in controlling stem rot of sesame. Proc. 8 Bangladesh Ann. Sci. Conf. Abs. p. 130.
- Islam, M. S. (1997). Performance of okra for vegetable and seed production in off- season sowing. M. S. Thesis. IPSA. Gazipur. pp. 39-41.
- ISTA. (2006). International Rules for Seed Testing. International Seed testing Association. Basserdorf, Switzerland.

- Jamader, M.M., Ashok, S., Shamro, J., Sajjan, A. and Jahangidar, S. (2001). Studies on seed mycoflora and their effect on germination of color graded okra [Abelmoschus esculents L.]. Crop Research Hiser. 22(3):479-484.
- Kant, K., Verrna, M. M. and Singli, S. (1999). Studies on the quality of vegetable seeds in the markets of Madhya Pradesh. Seed Res. 27(1):
 1-6.
- Khanzada, A. E., Sultana, N., Khan, S. and Aslam, M. (1988). Seed mycoflora of vegetables and its control. *Pakistan J. Sci. Indus. Res.* 31(8): 574-576.
- Kapri, B., Sengupta, A. K., De, B. K., Mandal, A. K. and Basu, R. N. (2003).
 Pre- storage seed invigoration treatments for improved germinability and field performance of okra. *Indian J. of Agril. Sci.* 73(5): 276-279.
- Lambat, A. K., Nath, R., Mukewar, P. M., Majumdar, A., Rani, I. and Chandra,
 K. J. (1974). Die-back diseases of bhendi (*Abelmoschus esculentus*. L.)
 Moench. Vegetable Sci. 1(1): 32-63.
- Majid, M. A. (1996). Seed health status of okra of two Sadar Thana of Mymensingh and Thakurgaon. M. S. thesis, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. p.104.
- Malakar, P.K., Mian, I. H., Bhuiyan, K. A., Akanda, A. M. and Reza, M. M. A. (2008). Effect of storage containers and time on seed quality of wheat. Bangladesh J. Agri. Res. 33:469-477.

- Mathur, S. B. and Kongsdal, O. (2003). Common Laboratory Seed Health Testing Methods for Detecting Fungi. Basserdorf, Switzerland. pp. 89-26.
- Mew, T. W. (1997). 'Seed health testing: Progress towards 21St Century'. In: Hutchins and Ruces (eds.), Development of rice seed health testing policy. pp: 129-138.
- Moretto, K. C. K., Barreto, M. And Inoue, R.Y. (1977). Survey of fungi associated with some vegetable seeds. *Horticulture Brasilera*. **15**(1): 7-10.
- Narayana, D. S. (1978). Seed Pathology. Vikash publishing house Pvt. Ltd. p. 62.
- Naseema, A., Balakrishnan, S. and Nair, M. C. (1983). Pathology and control of seed mycoflora of some vegetables in Kerala. Agril. Res. J. Kerala, 21(2): 32-37.
- Nema, N. P. (1986). Principles of Seed Certification and Testing. Allied Publishers Ltd., 13/14. Asraf Ali Rd., New Delhi.
- Neergaar, P. (1979). Seed pathology, Vol. 1. The Macmillan Press Ltd., London. p. 839.



- Pabitra, Kr. Bhanderi. (2008). A study on seed crop management of Okra for disease free and quality seed production. Ph.D. Thesis. Department of Plant Pathology. Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur. p. 157.
- Pandey, U. C. and I. J. Singh. (1979). Effect of nitrogen, plant population and seed moisture regimes on seed production of okra (Abelmoschus esculentus L. Moench). Vegetable Sci. 6 (2): 81-91.
- Pandita, V. K. and Randhawa (1992). Seed quality in relation to seed size in Radish. Seed Res. 20 (1): 47-48.
- Prasad, B. K., Sudhir, R., Manoj, k., Randheer, K., Kumar, S. K., Singh, K. R., Ranjan, S., Kurnar, M. and Kumar, R. (2000). Storage fungi of lady's finger seed and their significance. J. Phytol. Res. 13(1): 65-68.
- Pun, K. B., Sabitha, D., Valluvaparidasan, V. and Doraiswamy, S. (1998). Studies on seed-borne nature of *Macrophomina phaseolina* in okra. *Plant Dis. Res.* 13(2): 162-164. Vegetables and its control. *Pakistan J. Sci. Indus. Res.* 31(8): 574-576.
- Quayum, M. A. (1999). Influence of date of harvest on the incidence of seed borne fungal pathogens in okra. M.S. thesis, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. pp. 1-55.
- Rabman, M. and Mia. M. A. T. (1998). Studies on the health status of farmers stored seed of rice. *Bangladesh J. Plant Pathol.* 14(1 & 2): 37-40.

- Rashid, M. M. (1999). Sabjibiggan (in Bengali). Second edition. Rashid Publishing House. Dhaka. p. 526.
- Rashid, M. M. (2000). A Guide Book of Plant Pathology. Dinajpur, Bangladesh. pp. 86-89.
- Ribeiro, R., Del, D., Robbs, C. F., Akiba, F., Kimura, O. And Sudo, S. (1971). Studies on pre and post emergence rot of a okra (*Hibiscus esculentus*) in the lower carioca Fluminense region caused by a new special form of *Fusarium solani* (Mart) Appeal Arquivos da universidade Federal Rural do Riode Jeneiro. 5(1): 9-13.
- Richardson, M. J. (1979). An annotated list of seed-borne diseases. Third edt. CAB Publications. CMI, Kew, Surrey, U.K. pp. 106-108.
- Richardson, M. J. (1990). An annotated list of seed borne disease (Fourth edition). The International Seed Testing Association. Zurich, Switzerland. pp. 183-184.
- Robbs, C. F., Ribeiro, R., Del, D., Akiba, F. And Sudo, S. (1977). Notes on the occurrence of Fusariosis of okra in the Baixada carioca Fluminense, Brazil. *Abstracts Tropical Agril.* 3: 12500.
- Sahoo, P. K. and Srivastava, A. P. (2002). Physical properties of okra seed. Biosystems Engineering, Mathura, India. 83(4): 441-448.

- Sharma, H.K., Singh, P., Singh, S. and Meher, H. C., (2007). Management of root knot nematode, *Meloidogyne incognita* and wilt fungus, *Fusarium* oxysporum complex on okra. *Pesticide Res. J.* New Delhi, India, 19(2): 176-179.
- Shepherd, H and Winston, S. L. (1999). Performance evaluation of hybrids of Okra (Abelmoschus esculentus L.) Moench. For growth and yielding during kharif season in Allahabad region. Bioved. 10:105-107.
- Srivastava. S. K. and Gupta, 1981. Abelmoschus esculentus L. a new host for Colletotrichum dematium. FAO, Plant protection bulletin. 31 (3):130.
- Suratuzzaman, M., Hossain, I. and Fakir, G. A. (1994). Control of seed borne fungi of two rice varieties with some plant extracts. *Progress. Agric.* 5(1):11-15.
- Thomson, J. R. (1979). An Introduction to Seed Technology. Thomson Lithu Ltd., East Kilbride, Scotland. pp. 1-87.
- Thompson, H. C. and Kelly, W. C. (1957). Vegetable crop. 5th Edition. McGraw Hill Book Co. Inc. New York.p. 563.
- Tindall, H. D. (1988). Vegetables in the Tropics. MacMillan Education Ltd., London. pp. 325-328.

Tripathy, P. Maity, T. K. and Patnaik, H. P. (2008). Field screening of open pollinated okra varieties against major pests in response to reduced level of chemical fertilizers and organic manures. J. Plant Protection Environment, India. 5(1): 38-44.

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- USDA. (1960). Index of plant diseases in the United States. Agricultural Handbook No. 165. Crop Research Division, Agricultural Research Service, United States Department of Agriculture. p. 301.
- Varies, A. and Agrawal, P. K. (1992). Effect of accelerated ageing in respiratory rate and pathway of Soybean (*Glycine max*) Seeds. *Indian J. Exp. Bot.* 30:445-447.
- Vossen, H. A. M. B. (1994). Controlling and certifying the quality of seed in Bangladesh Paper presented on the implementation of the seed policy for the development of seed industry. Organized by the Ministry of Agriculture, Dhaka Bangladesh. pp. 56-60.
- Woodstock, L.W. (1969). Seedling growth as a measure of seed vigour. Proc. International Seed Testing Association (ISTA), **34**(2):273-280.