STUDY ON EFFECT OF STORAGE CONDITIONS ON HEALTH STATUS OF OKRA SEEDS

RAZIA SULTANA

DEPARTMENT OF PLANT PATHOLOGY

SHER-E-BANGLA AGRICULTURAL UNIVERSITY

DHAKA-1207

December, 2013

STUDY ON EFFECT OF STORAGE CONDITIONS ON HEALTH STASUS OF OKRA SEEDS

BY

RAZIA SULTANA

REGISTRATION NO : 12-05229

A thesis

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

MASTER OF SCIENCE (MS)
IN
PLANT PATHOLOGY

SEMESTER : JULY-DECEMBER, 2013

Approved by

Prof. Dr. M. Salahuddin M. Chowdhury	**Prof. Dr. Md. Rafiqul Islam**
Supervisor	Co-supervisor

Prof. Dr. F. M. Aminuzzaman
Chairman
Examination Committee
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207, Bangladesh

Dr. M. Salahuddin M. Chowdhury
Professor

Department of Plant Pathology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar
Dhaka-1207, Bangladesh
Fax: +8802-9112649
E-mail: smc_1968@yahoo.com

CERTIFICATE

This is to certify that thesis entitled, **"Study on Effect of Storage Conditions on Health Status of Okra Seeds."** submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY,** embodies the result of a piece of *bona fide* research work carried out by **RAZIA SULTANA**, Registration No. 12-05229 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 acknowledged.

Dated: 20 November, 2014
Dhaka, Bangladesh

(Dr. M. Salahuddin M. Chowdhury)
Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207
Supervisor

DEDICATED

TO

MY BELOVED HUSBAND

ACKNOWLEDGEMENTS

The author would like to express her deepest sense of gratitude, endless praises and thanks to the Almighty Allah for dealing His to get this far and for making all these possible, the Cherisher and Sustainer of the world. His kind blessings have enabled her to complete the paper.

The author wishes to express her heartfelt gratitude to **Dr. M. Salahuddin M. Chowdhury**, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, for his constant supervision, valuable suggestion, scholastic guidance, continuous inspiration, constructive comments and immense help for writing this manuscript from its embryonic stage.

The author wishes expresses her sincere appreciation, respect and immense indebtedness to respected co-supervisor Prof**. Dr. Md. Rafiqul Islam**, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, for cordial suggestions, constructive criticisms and valuable advice to complete the thesis.

The author would like to express special thanks to honorable Prof**. Dr. F. M. Aminuzzaman**, Chairman, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 for precious inspiration and guidance while working as a Full bright scholar at Sher-e-Bangla Agricultural University, constructive criticism and heartiest support.

The author would like to express her deepest respect to all the respected teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 for the valuable teaching, co-operation and inspirations throughout the course of this study and research work. The author wishes to express her cordial thanks to departmental and staffs for their active help during the experimental period. The author also sincerely expresses her heartfelt gratitude to the members of the advisory committee for partial financial support for conducting the research.

The author would like to express special thanks to Dr. Razab Ali, Professor, Department of Entomology, Khadija Akter, associate Professor, Department of Plant Pathology Sher-e-Bangla Agricultural University, Dhaka-1207 for his heartiest assistance in her research period.

Last but not least, she gratefully acknowledges her beloved parents and mother-in law, also obliged to her husband, her son & daughters, dearest classmates and other relatives and the well-wishers for the countless blessings, spiritual support and sacrifice throughout her academic life.

December, 2013 ***The Author***
SAU, Dhaka.

CONTENTS

Chapter	Title	Page No
	Acknowledgements	**i**
	Contents	**ii**
	List of Tables	**v**
	List of Figures	**vii**
	List of Appendices	**xi**
	Abbreviations	**x**
	Abstract	**xii**
I	**INTRODUCTION**	**1**
II	**REVIEW OF LITERATURE**	**7**
	2.1 Effects of storage containers and seed treatmen quality of okra seed	6
	2.1.1 Effect of containers on quality characters of seed	6
	2.1.2 Effect of seed treatment on quality characters of s	7
	2.1.3 Effect of storage containers and seed treatment on quality characters of seed	11
	2.2 Seed borne fungi associated with okra seeds and pathogenic effects	12
	2.3 Effect of containers for controlling the associated fungi of okra seed	18
	2.4 Effect of seed treatment for controlling the assoc fungi of okra seed	19

III MATERIALS AND METHODS 22

3.1 Experimental site 22

3.2 Experimental period 22

3.3 Atmospheric conditions of the storage room 22

3.4 Seeds collection 23

3.5 Experimental materials 23

3.5.1 Seeds of variety 23

3.5.2 Treatments 24

3.6 Experimental design and layout 25

3.7 Preparation and application of post-harvest treatments 25

3.8 Parameter study as quality tests 26

3.9 Method of studying characteristics 27

3.9.1. Moisture content 27

3.9.2 Germination test 28

3.9.3 Shoot length of seedling 28

3.9.4 Root length of seedling 28

3.9.5 Seedling vigor 29

3.9.6 Dry weight of seedling 29

3.9.7 Field emergence of seedling 30

3.9.8 Seed health test 30

3.9.8.1 Prevalence of fungi associated with randomly selected okra seeds 30

3.9.8.2 Pathogenicity test 32

3.10 Analysis of data 33

IV	**RESULTS**		**34**
	4.1	Evaluation of quality attributes of okra seed as influence by storage containers and seed treatments	34
	4.1.1	Moisture content (%) of okra seed	34
	4.1.2	Germination (%) of okra seed	36
	4.1.3	Shoot length of seedling	39
	4.1.4	Root length of seedling	40
	4.1.5	Seedling vigor index (SVI)	43
	4.1.6	Dry weight of seedling (g)	45
	4.1.7	Field emergence	46
	4.2	Prevalence of seed borne fungi of okra seed during the storage as influence by storage containers and seed treatments	49
	4.2.1	Prevalence of *Fusarium* spp	50
	4.2.2	Prevalence of *Chaetomium globosum*	52
	4.2.3	Prevalence of *Aspergillus flavus*	56
	4.2.4	Prevalence of *Aspergillus niger*	58
	4.2.5	Prevalence of *Rhizopus stolonifer*	62
	4.2.6	Prevalence of *Curvularia* spp	65
V	**DISCUSSION**		**70**
Vl	**SUMMARY AND CONCLUTION**		**74**
	REFERENCES		**76**
	APPENDICES		**88**

LIST OF TABLES

Table	Name of the Table	Page No.
1	Interaction effect of different storage containers and seed treatments on percentage of moisture content and germination of okra from the seed of different months of storage	42
2	Effect of different storage containers on length of shoot and root of okra seedling from the seed of different months of storage	44
3	Effect of seed treatments on length of shoot and root of okra seedling from the seed of different months of storage	45
4	Interaction effect of different storage containers and seed treatments on length of shoot and root of okra seedling from the seed of different months of storage	46
5	Effect of different storage containers on seedling vigor index and dry weight of seedling from the seed of different months of storage	47
6	Effect of seed treatments on seedling vigor index and dry weight of seedling from the seed of different months of storage	48
7	Interaction effect of different storage containers and seed treatments on seedling vigor index and dry weight of seedling from the seed of different months of storage	49
8	Interaction effect of different storage containers and seed treatments on field emergence of seedling from the seed of different months of storage	53
9	Interaction effect of different storage containers and seed treatments on the prevalence of *Fusarium* spp and *Chaetomium globosum* during the different period (months) of seed storage	59
10	Effect of different storage containers on the prevalence of *Aspergilus flavus* and *Aspergilus niger* during the different period (months) of seed storage	64

11	Effect of seed treatments on the prevalence of *Aspergilus flavus* and *Aspergilus niger* during the different period (months) of seed storage	64
12	Interaction effect of different storage containers and seed treatments on the prevalence of *Aspergilus flavus* and *Aspergilus niger* during the different period (months) of seed storage	65
13	Effect of different storage containers on the prevalence of *Rhizopus stolonifer* during the different period (months) of seed storage	67
14	Effect of seed treatments on the prevalence of *Rhizopus stolonifer* during the different period (months) of seed storage	68
15	Interaction effect of different storage containers and seed treatments on the prevalence of *Rhizopus stolonifer* and *Curvularia* during the different period (months) of seed storage	72

LIST OF FIGURES

No	Name of the Figure	Page No
1	Collected okra seed (BARI Dherosh–1)	23
2	Photograph showing different container of storage; A) Polythene coated gunny bag (pgb), B) Gunny bag (gb), C) Plastic bag (pb), D) Plastic container or pot (pc) and E) Earthen pot (ep)	24
3	Seedlings germinated in paper towel method	29
4	Seed health test by blotter method	31
5	Rolled paper towel method	32
6	Pathogenicity test	33
7	Raising of seedlings	33
8	Effect of different storage containers on percentage of moisture content of storage okra seed at different months of storage	35
9	Effect of seed treatments on percentage of moisture content of storage okra seed at different months of storage	35
10	Effect of different storage containers on percentage of seed germination of okra seed at different months of storage	37
11	Effect of seed treatments on percentage of seed germination of okra seed at different months of storage	37
12	Effect of different storage containers on field emergence of seedling (%) of okra at different months of storage	48
13	Effect of seed treatments on field emergence of seedling (%) of okra at different months of storage	48

14	Effect of different storage containers on the prevalence of *Fusarrium spp* in storage okra seeds (%) at different months of storage	51
15	Effect of seed treatments on the prevalence of *Fusarrium* spp in storage okra seeds (%) at different months of storage	51
16	*Fusarium spp* infected okra seed	52
17	Conidiophores and conidia of *Fusarium spp* under	52
18	Effect of different storage containers on the prevalence of *Chaetomium globosum* in storage okra seeds (%) at different months of storage	54
19	Effect of seed treatments on the prevalence of *Chaetomium globosum* in storage okra seeds (%) at different months of storage	54
20	Chaetomium globosum under compound microscope	56
21	*Chaetomium globosum* under compound microscope	56
22	*Aspergillus flavus* infected okra seed	58
23	Conidiophores and conidia of *Aspergillus flavus* under compound microscope	58
24	*Aspergillus niger* under stereomicroscope	62
25	Conidia and conidiophore of *Aspergillus niger* under compound microscope	62
26	Sporangia of *Rhizopus* spp isolated from okra seed	65
27	*Curvularia* spp infected okra seeds under stereo –microscope	66
28	Effect of different storage containers on the prevalence of *Curvularia* spp in storage okra seeds (%) at different months of storage	67
29	Effect of seed treatments on the prevalence of *Curvularia* spp in storage okra seeds (%) at different months of storage	67
30	Conidia, conidiophores and mycelia of *Curvularia* spp	69

LIST OF APPENDICES

I	Analysis of variance for moisture content and germination percentage of okra from the seed of different months of storage (MAS) as influence by storage containers and seed treatment	88
II	Analysis of variance for length of shoot and root of okra seedling from the seed of different months of storage (MAS) as influence by storage containers and seed treatment	88
III	Analysis of variance for seedling vigour index and dry weight of okra seedling from the seed of different months of storage (MAS) as influence by storage containers and seed treatment	88
IV	Analysis of variance for field emergence of okra seedling from the seed of different months of storage (MAS) as influence by storage containers and seed treatment	89
V	Analysis of variance for the prevalence of *Fusarium* spp and *Colletotrichum* spp in okra seed at different months of storage (MAS) as influence by storage containers and seed treatment	89
VI	Analysis of variance for the prevalence of *Aspergilus favus* and *Aspergilus niger* in okra seed at different months of storage (MAS) as influence by storage containers and seed treatment	89
VII	Analysis of variance for the prevalence of *Rhizopus stolonifer* and *Curvularia* spp in okra seed at different months of storage (MAS) as influence by storage containers and seed treatment	90
VIII	Atmospheric conditions of the storage room	90

ABBREVIATIONS

%	: Percentage
°C	: Degree Centigrade
µM	: Micro mol
AEZ	: Agro-Ecological Zone
Anon.	: Anonymous
AWD	: Alternate Wet-Dry
BARC	: Bangladesh Agricultural Research Council
BARI	: Bangladesh Agricultural Research Institute
BBS	: Bangladesh Bureau of Statistics
BRAC	: Bangladesh Rural Advancement Committee
BRRI	: Bangladesh Rice Research Institute
CARE	: Co-operations for American Relief Everywhere
cm	: Centimeter
CRD	: Completely Randomized Design
CV (%)	: Percent Coefficient of Variance
cv.	: Cultivar
DAE	: Department of Agricultural Extension
DAS	: Days After Sowing
DAT	: Days After Transplanting
DMRT	: Duncan's Multiple Range Test
dw	: Distilled water
e.g.	: Exempli gratia (by way of example)
e.g.	: *exempli gratia* (L), for example, any other
etc.	: Etcetera
FAO	: Food and Agriculture Organization

Fig.	: Figure
g	: Gram
GA_3	: Gibberellic acid
HI	: Harvesxzt Index
i.e.	: *id est* (L), that is
IRRI	: International Rice research Institute
LSD	: Least Significant Difference
MAS	: Month after storage
MC	: Moisture content
m^2	: Meter squares
mgL^{-1}	: Milligram per litre
NGO	: Non-Governmental Organization
NSB	: National Seed Board
pH	: Negative logarithm of hydrogen ion concentration
SAU	: Sher-e-Bangla Agricultural University
spp	: Species (plural number)
SRDI	: Soil Resource Development Institute
t ha^{-1}	: Ton per hectare
UNDP	: United Nations Development Programme
var.	: Variety
Viz.	: Namely

STUDY ON EFFECT OF STORAGE CONDITIONS ON HEALTH STATSUS OF OKRA SEEDS

ABSTRACT

An experiment was conducted in Seed Pathology Lab in the Department of Plant Pathology, SAU, Sher-e-Bangla Nagar, Dhaka-1207 during July 2013 to June 2014 to determine the quality and health of okra seed and determination of seed health status during seed storage. Five storage container *viz.* T_1: plastic container, T_2: plastic bag, T_3: polythene coated gunny bag, T_4: gunny bag and T_5: earthen pot and three seed treatments *viz.* N_0: no treatment, N_1: seed treated with Provax-200 and N_2: seed treated with neem leaf powder extract were tested. Data were recorded on the percent infestation of storage seeds by fungi and also on quality attributes under different treatments during the seed storage at 4, 8 and 12 months. The experiment was laid out in two factors Completely Randomized Design (CRD) with three replications and the mean were adjusted by DMRT at 5% level. In case of storage container and seed treatments, provax-200 treated seeds stored in plastic container (T_1N_1) showed significantly lower moisture content (7.98%) at 4 months of storage than that of other treatments while non - treated seeds stored in gunny bag (T_4N_0) showed the maximum moisture content (17.66%) at 12 months of storage. Similarly, percentage of seed infested minimum in T_1N_1. The prevalence of different fungi were *Fusarium* spp (0.13%), *Chaetomium globosum* (0.72%), *Aspergillus flavus* (0.52%), *Aspergillus niger* (0.87%), *Rhizopus stolonifer* (0.59%) *and Curvularia* spp (0.80%) at 4 months of storage. Seed infested was highest in T_4N_0 where prevalence of *Fusarium* spp (9.27%), *Chaetomium globosum* (11.96%), *Aspergillus flavus* (18.26%), *Aspergillus niger* (16.63%), *Rhizopus stolonifer* (8.72%) *and Curvularia* spp (8.20%) at 12 months of storage. In contrast, provax-200 treated seeds stored in plastic container (T_1N_1) showed the maximum germination (96.74%), longest shoot (21.04 cm) and root (11.91 cm), highest seed vigor index (3229.0), highest weight of dry seedlings (55.72 g) at 4 months of storage while non-treated seeds stored in gunny bag (T_4N_0) showed lower performance in seed germination (39.71%), shoot length (11.33 cm), root length (6.20 cm), seedling vigor index (731.30), dry weight of seedlings (33.33 g) and filed emergence (34.51%) at 12 months of storage in this study. The above result proved that the studied attributes considerably increase with increasing storage period except seed germination, seedlings shoot and root length, seed vigor index, dry weight of seedlings and field emergence where plastic container (T_1) and seed treated with provax-200 (N_1) singly or their interaction effect had highly efficient for obtaining the greater performance regarding quality attributes of storage okra seed and also for controlling the fungi species. The storage plastic container along with provax-200 treated seeds showed the best storage performance in storage of okra seed.

CHAPTER I

INTRODUCTION

Okra, *Abelmoschus esculentus* (L.) Moench, is an important vegetable crop grown mainly in the tropical or sub-tropical regions during summer and rainy season. Hence, it is classified as a warm season crop (National Research Council, 2006). The major okra producing countries in the world include India (3.5 million tons), Nigeria (0.73 million tons), Pakistan (0.12 million tons), Ghana (0.10 million tons) and Egypt (0.08 million tons) (Nwangburuka, 2010; Badaru, 2011). It is regarded as an important vegetable throughout the country including the south-east areas of Bangladesh. Though it is grown round the year, its production is mainly concentrated during summer season in Bangladesh. It is locally known as "Dherosh" or "Bhendi" which belongs to the family Malvaceae (Sarkar, 2010). It is grown commercially in India, Turkey, Iran, Western Africa, Yugoslavia, Bangladesh, Afghanistan, Pakistan, Burma, Japan, Malayasia, Brazil, Ghana, Ethiopia, Cyprus and the Southern United States. In this context Aladele *et al.,* 2008 collected 93 accessions of okra comprising of 50 West African genotypes (*A. caillei*) and 43 Asian genotypes (*A. esculentus*) and assessed for genetic distinctiveness and relationship using random amplified polymorhphic DNA (RAPD). In Bangladesh, the total vegetable production of Bangladesh (summer and winter) is 30,68,000 metric tons with an average yield of 3,378 kg/acre under the total vegetable cultivation are of 9,08,000 acres while 26,000 acres was used for okra cultivation with an average yield of 1,680 kg/acre and the total production is 43,000 metric tons during 2010-2011 growing season (BBS, 2012). The yield of okra though is not quite high compared to other okra growing countries.

Okra seeds contain a considerable amount of good quality oil and protein and can be used as a substitute for coffee (Valeriana, 2002). It has been found that okra

mucilage application serves as a plasma replacement or blood volume expander (Onunkun, 2012). 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). Besides, Gopalan *et al*. (2007) reported that in 100 g okra seeds contains 35.0 g calories, 89.6% moisture, 6.4 g carbohydrates, 1.9 g protein, 0.2 g fat, 1.2 g fiber, 0.7 g minerals, 56.0 mg phosphorus, 6.9 mg sodium, 30.0 mg sulphur, 66.0 mg calcium, 0.35 mg iron, 103.0 mg potassium, 53.0 mg magnesium, 0.19 mg copper and 8.0 mg oxalic acid. The edible portion of the fruit, on average, contains approximately; 86.1% moisture, 9.7% Riboflavin, 0.01 mg carbohydrates, 2.2% protein, 1.0% fibers, 0.2% fats and 0.9 % ash. The ripe seeds contain Thiamine (0.07 mg), approximately 20% edible oil. Okra is a good source of vitamins A, B, C and minerals, (Nicotinic acid 0.06 mg) especially Iodine.

Various factors are responsible for low yield of okra. Seed-borne fungal diseases are often the main cause. In most regions of the world, okra crop is produced in large quantities, poor agronomic practices and storage conditions including improper drying and inadequate structures have contributed to the reportedly high prevalence of fungi contaminants of okra especially seed-borne molds. Fungi are one of the most important and prevalent pathogens of okra and they usually attack the crop from seedling to harvesting. Most fungal diseases of okra during post-harvest storage are usually caused by residuals of fungi spores which are transferred from the field and develop into disease during storage. Fungi that affect okra seeds include; *Alternaria alternate*, *Aspergillus niger*, *Curvularia lunata*, *Fusarium oxysporum, F. solani*, *F. moniliforme*, *Macrophomina phaseoli*, *Rhizoctonia*, *Stemphylium botryosum*, *Penicillium digitatum*, *Pythium aphanidermatum* (Al-Kassim and Monawar, 2000, Odofin, 2010). Major seed-borne diseases of okra in Bangladesh are seed rot, seedling blight, die back and

anthracnose caused by *Chaetomium globosum*: 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. spp *vasinfectum* (Fakir, 2001). Among these fungal pathogens *Chaetomium globosum* and *Macrophomina phaseolina* are both seed transmitted. Plant pathogens like fungi, bacteria, virus, and viroids are carried ‘‘in’’, ‘‘on’’ or ‘‘within’’ the seeds and as a result, these pathogens are responsible for causing heavy crop losses. Nearly 231 virus and viroid diseases are seed-borne in different plants (Sastry 2013).

For the management of seed transmitted diseases storage container and seed treatment is an important measure. It is unquestionable that suitable storage container and proper seed treatment can substantially improve the quality of seed and seedling with satisfactory increase in the yield. Storage container and seed treatment may probably be the cheapest and safest method of plant disease control. Therefore along with routine seed health testing, suitable storage container for seed storage and proper seed treatment before sowing is necessary. In many countries regular practice of storage container and seed treatment is considered as insurance against the building up of inoculate in the environment and has greatly reduced the yield loss and improves seed quality in many crops.

Adebisi and Oyekale, (2005) reported that germ inability of okra seed inside different containers stored under ambient conditions for 6 months showed a downward decline in viability. Oliveira *et al*. (2011a) and Silva *et al*. (2011) reported that adequate storage can maintain higher seed viability and vigor than would normally be possible under natural conditions and the types of packaging materials used, the temperatures and relative humidity of the storage environments play important roles in maintaining seed physiological quality. Germination studies with *C. adamantium* seeds were undertaken by Melchior *et al*. (2006) and

Scalon *et al*. (2009) although their results, especially in terms of their ideal storage times, seed water contents and the environmental conditions and packaging materials necessary to conserve their physiological quality are somewhat contradictory suggesting that the seeds are sensitive to desiccation and to storage procedures (Zaidan; Carreira, 2008). Several authors, Adebisi and Oyekale (2005), Abdui-Rafiu (2007) and Esuruoso (2010) have reported the effectiveness of apron (packing materials) plus seed treatment chemical on the maintenance of seed germination and seedling vigor in soybeans and okra. Furthermore, the fungicide itself may be degraded during storage and may become ineffective after certain storage time (Adebisi *et al.,* 2003). Islam (2006) also reported that the proper storage condition and storage containers can maintain the seed health status as well as seed viability and vigor while air tight or sealed container is the best for okra seed storage (Islam, 2006).

In the past, researchers explored the use of inorganic chemicals (e.g. fungicides) in post-harvest crop/seed protection against pathogens. Fungicides such as azoxystrobin, mefenoxam and several others have been registered for use against seed pathogens in okra (Mossler and Dunn, 2009). Recently, okra seeds have been treated with disinfectants like bleach and natural plant extracts such as neem and *Moringa* (Nwangburuka *et al.,* 2012). Odofin (2010) reported that neem extract inhibited the vigor and induced some chromosomal aberrations in okra seeds by seed bleach which increased germination and vigor. Odofin (2010) also reported that the seed quality deteriorates during post-harvest storage as a result of pest and pathogen infestation and poor storage condition. Hence, post-harvest treatments of seed are necessary to control seed losses during storage. However, treatments of seeds with chemicals are quite effective in reducing seed–borne infection. But uses of chemicals are hazardous and costly. However, most of our farmers use seed treating chemicals (e.g. Vitavax–200, Bavistin 50 wp, provax-200 etc.) to control seed–borne pathogens. There are reports about some botanicals like garlic, neem, allamanda

extracts and some bio control agents like *Trichoderma harzianum*, which can be effectively used as a seed treating agent as an alternative to chemicals. An appreciable amount of work has been done on the control of seed–borne pathogens of okra and other crops with fungicides (Vannacci *et al*., 2001; Patel *et al*., 2004). Seed treatment with plant extracts and bio–fungicide is developing and not enough works have been done in Bangladesh (Chowdhury *et al*., 2000 and Akter, 2001).

Most of the farmers of our country have limited knowledge on storage procedure of okra seeds while farmer can easily established the healthy plant to enhance the production of okra in Bangladesh. Besides, they also do not known how to increase the efficiency and longevity of okra seed as well the conformity of healthy seeds. From the above facts, it was clear that the storage container and plant extracts had shown good results as storage materials for longevity of okra seeds maintaining good quality. Considerable amount of study have been done with chemical fungicide to control seed-borne disease of okra (Akter, 2008 and Ahmed, 2011) and storage container as well as packing materials for keeping the quality for long time during storage. But a few studies were done to control the seed-borne fungi of Okra using plant extracts. But management of these seed-borne fungi is very important to produce okra successfully. In view of the above facts, the present study was undertaken to achieve the following objectives.

i. To determine the seed quality and prevalence of seed–borne fungi on okra seed;
ii. To observe the effect of storage containers and duration of storage period on seed quality and prevalence seed–borne fungi on okra seed and;
iii. To identify the best performing seed treating agent on seedling vigor and prevalence of seed–borne fungi during storage.

CHAPTER II
REVIEW OF LITERATURE

Very little work has been on the quality observation of okra seed followed on germination and seedling test and also the association of seed borne fungal pathogens during the storage as influenced by the effect of storage containers and seed treatment during storage.

2.1 Effects of storage containers and seed treatments on quality of okra seed

2.1.1 Effect of containers on quality characters of seed

Aktaruzzaman, *et al*. (2010) studied the effect of initial moisture content and different storage containers on the quality of okra seeds namely sealed container (C_1), polythene bag (C_2) and gunny bag (C_3) which revealed that the germination percentage of okra seeds of sealed container was the highest (69.48%) and significantly vary from poly bag and gunny bag.

Fabunmi (2009) studied the effect of packaging materials on moisture contents of okra (*Abelmoschus esculentus*) at room (28+/–2 deg C) and refrigeration temperatures (15+/–2 deg C) using three different packages (open plastic bowl as control, plastic sieve over–wrapped with low density polyethylene bags and low density polyethylene bags (LDPE)–15 x 15 cm). The results showed that packaging materials had a significant effect on moisture content where okra stored in polyethylene followed by plastic sieve container controlled moisture content.

Begum, *et al*. (2005) reported that the Okra is susceptible to several fungal pathogens which resulted in the reduction of yield and nutritional quality. Healthy, moderately and severely infected seeds obtained from local market were evaluated for their storability for a year using different storage containers (cotton bag,

polyethylene and paper bags) at varied temperatures. Results indicated that the moisture content play a key role in amplifying fungal biomass during storage period. Cotton bag at 28^{o}C appeared to be the best for long storage of seeds and in respect to fluctuation in moisture content, cotton bags offered greater protection than that of polyethylene or paper bags.

Ghimire (2003) studied the effects of different storage periods and packaging materials on storability of onion and okra seeds stored in different type of packaging materials significantly influenced the normal seedling production (standard germination), seedling emergence (viability) and speed of germination (vigor). It was found that after 9 months of storage, the mean germination percent was the highest in laminated pouches (70.81%) followed by plastic containers (68.50%), polyethylene bags (67.38%), plastic sacks (57.88%) and cloth bags (57.13%) in case of okra and also stated that the seed qualities were maintained for longer period in laminated pouches, plastic containers and polyethylene bags.

Doijode (1997) reported that the tomato and okra seeds had maintained the viability up to four years and two years respectively, when stored in poly bags of 700 guage under ambient temperature of 16–35^{o}C as compared to sub–zero temperature of –2^{o}C.

Saxena (1994) concluded that polythene bags of 700 guage could be used for long term storage of various vegetable seeds like onion, tomato, okra and cabbage which were with six percent (6%) moisture content.

2.1.2 Effect of seed treatment on quality characters of seed

Salvador, *et al*. (2013) performed germination, seedling emergence, controlled deterioration (seeds with moisture contents of 18, 21 and 24% at 45°C for 24 and 48 hours) and moisture content tests and reported that the combination of 24%

water, 45°C during 24 hours was recommended to cause potential physiological deterioration of okra seeds.

Keshavulu, *et al*. (2012) investigated the accelerated ageing at 40 + 1^0C and 85 + 5 % relative humidity for 4, 8, 12 and 16 days to study the effect of seed vigor on initial seed quality and field performance. The initial seed quality declined with increase in period of accelerated ageing. Plants established from low vigor seed lots exhibited poor field performance as evidenced by reduced plant height, reduction in dry matter production and leaf area per plant at different stages of crop growth.

Adebisi (2012) evaluated the efficacy of four organic crude plant materials, three inorganic chemicals and hydration, dehydration seed treatments on the maintenance of germination, seedling vigor and seed longevity of stored freshly harvested okra seeds under natural ageing in ambient condition of seed storing for 180 days. Among the treatments, significant differences were observed for seed germination and seedling vigor. Seeds dressed with apron–plus and crude ocimum leaf powder gave better seed germination with an average improvement between 0 and 12% over control while other seed treatments gave significant higher values above their respective controls. On the storage effect, seed dressed with ocimum and apron–plus powders were the most effective in storage as it consistently maintained superior seed germination over 180 days of storage while ocimum and mango leaf treated seeds consistently retained higher seedling vigor at each storage time than other treatments. Pre storage treatments of freshly harvested okra seeds with fine ocimum leaf powders (active ingredient: linalool) at 10gm per 100gm seed is therefore advocated for overall maintenance of vigor and germination of okra seed. The study also recommended that the use of plant crude materials may be considered as effective seed storage management approach.

Fagbohun and Faleye (2012) studied six fungi namely: *Penicillium* spp, *Mucor* spp, *Aspergillus niger*, *Aspergillus flavus* and *Neurospora crassa* and found to increase those fungi when the storage time increased.

Guha, *et al*. (2012) reported that the field performance and productivity of the pre and mid storage dry treated seeds, especially with bleaching powder, red chilli powder and aspirin were significantly greater than that of untreated (control) seed. Thus, pre–storage dry seed treatments with red chilli powder and bleaching powder as well as mid storage soaking–drying treatment were suggested for the maintenance of germinability during storage and field performance of okra.

Islam (2012) tested the effect of extracts of Garlic, Allamanda and Neem to control seed borne fungi of Okra. In treated seeds among all the plant extracts viz. garlic, neem and allamanda germination ranged from 60–90%. Garlic extracts @ 1:1 showed best performance in increasing seed germination (95.5%) followed by Neem (60%) and reduction of fungal flora (53.25%). Vigor index of Okra seeds increased 27.91% over untreated seeds by the treatment of Garlic extracts @ 1:1 and it seemed to be adoptable at the farmer's level as an organic management practice.

Roy, *et al*. (2012) experimented on aqueous extracts of leaves of some herbal plants viz., *Beleric myrobalan*; bohera (*Terminalia belirica*), *Chebulic myrobalan*; horitoki (*Terminalia chebula*), Sweet basil; tulsi (*Ocimum gratissmum*) and A*rjunaa myrobalan*; arjun (*Terminalia arjuna*). The aqueous extract of bohera leaves increased germination of seeds tested and enhanced the growth of shoot length and root length of okra (*Hibiscus esculentus*). The maximum germination was in okra seeds within 5 and 4.8 days respectively, treated with the leaves extract of bohera. Shoot lengths was 16.2 cm and root length was 8.74 cm for okra seedlings. The aqueous extract of horitoki showed the lowest rate and late germination at 6 and 5.8 days and minimum shoot and root lengths were 13.13 and 5.91 cm for okra seedlings.

Mashooda and Lokesh (2011) reported that okra seeds treated by aqueous leaf extracts of *Coleus aromaticus*, *Adathoda vesica*, *Vitex negundo*, *Solanum nigrum*, *Leucas aspera*, *Ocimum sanctum* and *Catharanthus roseus* resulted in increased seed germination and vigor of the seedlings. Both in green house and field conditions also these extracts proved their efficiency in the enhancement of biomass, number of leaves.

Dawar, *et al*. (2008) reported that biocontrol agents enhanced the germination and growth of plant in respect of shoot length, shoot weight, root length, root weight etc. which were significantly increased in both okra and sunflower. Maximum plant height was observed where seeds of okra and sunflower were coated with *T. harzianum* using 2% of glucose followed by gum arabic, mollases and sugar solution. Gum arabic was found more effective in reducing infection by root rot fungi viz., *M. phaseolina*, *R. solani* and *Fusarium* spp.

Abduhu (2007) investigated to determine the seed quality and seed health status of okra seeds in which moisture contents of seed samples ranged from 10.1 to 16.1% and germination percentages ranged 14 to 87 %.

Tariq, *et al*. (2006) observed that the use of leaves, stem and pneumatophore of *Avicennia marina* in controlling the root infecting fungi viz., *Fusarium* spp, *Macrophomina phaseolina* and *Rhizoctonia solani* on mash bean and okra plants and the germination of seeds, shoot length, root length, shoot weight and root weight significantly increased in both okra and mash bean where *A. marina* plant parts viz., stem and pneumatophore powder was used @ 5% w/w.

Islam (2006) studied the effect of different treatments (where, T_0= control, T_1= 1:1 dilution, T_2= 1:2 dilution and T_3= 1:3 dilution of neem extract and mehagoni) on germination. In case of neem extract, the maximum number of germinated seeds 70.25% was recorded in T_2 than all other treatments while minimum 54.50% was found in T_0.

Islam (2004) tested the effect of four different treatments (viz.- Furadan 3G, neem leaf extract, Furadan 3G combined with neem leaf extract and a control) on plant growth and a higher plant growth, with increased length of shoot and root and fresh weight of shoot and root with lower galling incidence and lower development of egg masses, adult females were recorded when plants treated with Furadan 3G either alone or in combination with neem leaf extract. Better response was found with neem leaf extract.

Anam, *et al*. (2002) studied the effect of seed treatment on the incidence of seed borne fungal diseases and on production of seed yield of okra. The lowest germination (95.0%) was recorded in unclean farmer's seeds; while highest germination (99.0%) was recorded in Vitavax–200 treated seeds followed by clean apparently healthy seeds (98.5%).

Rahman, *et al*. (2000) observed that seed treatment with Vitavax showed higher shoot and root length which was followed by manually cleaned seed and floatation method.

Dash and Narain (1996) observed that the pre–treatment of seeds of most crops (okra, cowpea, sorghum, wheat, *Vigna radiata* and *luffa acutangula*) with Bavistin + TMTD (carbendazim + thiram) considerably improved seed germination. For most of the test crops, Bavistin + TMTD, Thiram, Brassicol (quintozene), Difolatan (captafol), Dithane (Flowable) (mancozeb) and Vitavax (carboxin) improved seed germination with decreasing efficacy.

2.1.3 Effect of storage containers and seed treatment on quality characters of seed

Haque (2014) investigated nine different storage containers viz. tin pot, plastic pot, polythene bag, gunny bag, gunny bag lined with polythene, earthen pot, cloth bag,

brown paper, and IRRI poly bag; two levels of moister contents [viz. farmers content (16%) and recommended moister condition (9.5%) by Bangladesh Gazette in 2010] and seed treatment with provax-200 and found that seeds were stored for 12 months and examined after 4, 8 and 12 months of storage. Among the nine containers, tin pot was found better in respect of seed health status. The poorest performance was observed in earthen pot regarding seed health status. The findings also reveal that recommended moister content (9.5% moister content in seed) was better than moister contain at farmer's condition. Provax-200 treated seed also result better performance than control condition's seed. So, quality of jute seeds can be maintained by storage in tin pot with provax-200 treated and recommended moister content (9.5%).

2.2 Seed borne fungi associated with okra seeds and their pathogenic effects

Hamim, *et al*. (2014) evaluated the seed borne infection and germination of seeds of 7 vegetables namely amaranth, red amaranth, spinach, okra, cucumber, tomato and eggplant where they found that 11 fungi were detected which were *Alternaria* spp, *Aspergillus flavus*, *Aspergillus niger*, *Phomopsis* vexans, *Curvularia* spp, *Fusarium* spp, *Penicillium* spp, *Penicillium* spp, *Colletotrichum dematium*, *Macrophomina phaseolina* and *Cladosporium* spp. Six fungi were detected in amaranth, six fungi in red amaranth, four fungi in spinach, six fungi in okra, four fungi in cucumber, four fungi in tomato and five fungi in eggplant seeds. The highest total seed borne fungal infection was found in okra (26.75%), while the lowest was found in cucumber (13.50%).

Sharma, *et al*. (2013) reported that the okra is attacked by several pathogens during storage. Seventy eight fruit samples of okra from different sights i.e. market, farmers field and farm storage (houses) were collected and eleven major fungal species (*Aspergillus flavus* , *A. niger, A. nidulens, A. fumigates, Alternaria alternate, Curvularia lunata, Penicillium nigricans, Cladosporium oxysporum,*

Penicillium chrysogenum, *P. citrinum*, *Stachybotrus atra*, *Chaetomium globosum*, *C. murorum*, *Rhizoctonia bataticola*), and four bacterial genera (*Actinomycetes* spp, *Erwinia caratovora*, *Xanthomonas campestiris* pv. *campestris*, *Xanthomonas campestiris* pv. malvacearum, *Pseudomonas syringae* pv. syringae) were found associated with post harvested diseases or spoilage of okra fruits in the study. It was concluded that fungal pathogens cause the damage at high temperature, low relative humidity with poor aeration and bacterial damage at high humidity and low aeration. These pathogens showed 04–72 % loss due to said pathogens.

Mohanto (2013) studied seed borne infection and germination of seeds of 7 vegetables viz. Amaranth, Red amaranth, Spinach, Okra, Cucumber, Tomato and Brinjal where 11 fungi namely *Alternaria* spp, *Aspergillus flavus*, *Aspergillus niger*, *Phomopsis vexans*, *Curvularia* spp, *Fusarium* spp, *Penicillium* spp, *Penicillium* spp, *Colletotrichum dematium*, *Macrophomina phaseolina* and *Cladosporium* spp were detected and out of these, six fungi were detected in Amaranth, six fungi in Red amaranth, four fungi in spinach, six fungi in okra, four fungi in cucumber, four fungi in tomato and five fungi in Brinjal seeds. The highest total seed borne fungal infection was found in Okra (26.75%), while lowest was found in Cucumber (13.50%).

Sharma, *et al*. (2013) found that eighty nine seed samples of okra were evaluated and were found *Alternaria alternata*, *Arthrobotrys superba*, *Aspergillus flavus*, *A. niger*, *Cladosporium oxysporium*, *Curvularia lunata*, *Drechslera* spp, *Fusarium* spp, *Rhizoctonia bataticola* and *Penicillium nigricans* as fungal species and *Ralstonia solanacearum* (Smith) Yabuuchi *et al*., *Pseudomonas syringae* var. syringae van Hall and *Xanthomonas axonopodis* var. *malvacearum* (Smith) Vauterin as bacterial species. This microflora severely affected seeds germination and caused many seedling abnormalities like failure or delayed germination, bacterial oozing, stunting, rotting, wilting and puffing of seedlings, collapse of hypocotyls and cotyledonary leaves resulting seedling mortality.

Islam (2012) studied on the control of seed borne fungi of okra and found six predominant fungal genera namely *Fusarium oxysporum* (5.33%), *Aspergillus flavus* (4.3%), *Aspergillus niger* (5.30%), *Colletotrichum dematium* (4.80%), *Penicillium stolonifer* (3.66%) and *Penicillium* spp (3.17%).

Fagbohun and Faleye (2012) reported that the six fungi were isolated namely: *Penicillium* spp, *Mucor* spp, *Aspergillus niger*, *Aspergillus flavus* and *Neurospora crassa* during the study with okra seeds. The fungi were found to increase as the storage time.

Thippeswamy, *et al*. (2011) found the incidence of fungi associated in different vegetable crops viz., chilli, brinjal, tomato and okra, the incidence of fungi namely *Colletotrichum capsici, Alternaria alternata, Alternaria solani, Fusarium oxysporum, Fusarium solani, Phomopsis vexans, Macrophomina phaseolina*, *Aspergillus* spp and *Penicillium* spp were predominant on seed borne fungi and found to reduce seed germination.

Sultana (2009) studied health condition of country bean, tomato, okra, cowpea seeds and found eight fungi viz. *Aspergillus* spp, *Fusarium* spp, *Botrytis* spp, *Curvularia* spp, *Colletotrichum* spp, *Penicillium* spp and *Phomopsis* spp. Among the pathogens, *Aspergillus* spp was highly prevalent in all the crop seeds ranging from 1.60 % to 14 %.

Dawar, *et al*. (2008) found different microbial antagonists viz., *Bacillus thuringiensis, Rhizobium meliloti*, *Aspergillus niger* and *Trichoderma harzianum* active in case of the seeds coated with gum arabic, glucose, sugar and mollases in the suppression of root rot fungi viz., *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp in okra and sunflower plants.

Akter (2008) found *Colletotrichum dematium*, *Macrophomina phaseolina*, *Fusarium oxysporum*, *Fusarium moniliformae*, *Cercospora* spp, *Aspergillus niger*, *Aspergillus flavus* and *Penicillium* spp as seed borne fungi on okra seeds.

Abduhu (2007) observed eight fungi namely *Aspergillus flavus*, *A. niger*, *Fusarium oxysporum*, *Fusarium* spp, *Macrophomina phaseolina*, *Colletotrichum dematium*, *Curvularia* spp and *Chaetomium* spp to be associated with the seed samples of okra. Among the fungi prevalence of *Aspergillus* spp was maximal which was followed by *Fusarium* spp.

Mithal (2006) observed okra seeds to be attacked with different fungi such as *Macrophomina phaseolina*, *Rhizoctonia bataticola, R. solani*, *Fusarium solani*, *Pythium butleri, Phytophthora palmivora*, *Cercospora abelmoschii* and *Erysiphe cichoracearum* etc.

Karim (2006) studied fungal prevalence and their control and found eight different fungi namely *Colletotrichum dematium*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp and *Penicillium stolonifer* were predominantly associated with okra seeds.

Alam and Rashid (2005) reported eight fungi such as *Aspergillus* spp, *Penicillum* spp, *Curvularia* spp, *Fusarium* spp, *Penicillium* spp, *Colletotrichum* spp, *Alternaria* spp and *Macrophomina* spp were associated with the seeds samples of okra available in the market.

Gurjar, *et al*. (2004) conducted an experiment to manage the seed–borne pathogens of okra and found that the *Curvularia lunata*, *Macrophomina phaseolina*, *Fusarium oxysporum*, *F. moniliforme* and *F. pallidorosum* were associated with okra.

Jamandar, *et al*. (2001) reported that different colored okra seeds were associated with 27 fungi species and among them *Aspergillus flavus*, *Aspergillus niger*, *Colletotrichum gloeosporiodes* (*Glomerella cingulata*), *Fusarium moniliformae* (*Gibberella fujikuroi*), *Rhizoctonia solani*, *Penicillium nigricans* (*Penicillium stolonifer*) and *Phomopsis* spp were the predominant fungi. They found that black colored seeds had the highest percentage of seed germination was zero and 5% in black seeds harvested during Kharif and Rabi respectively.

Alam (2001) reported different pathogens from different vegetables seeds and *Aspergillus*, *Penicillium*, *Fusarium*, *Penicillium*, *Colletotricum*, *Alternaria* and *Macrophomina* were recorded from okra seeds.

Shahid, *et al*. (2001) studied the survival of seed borne inoculums and seed transmission of *M. phaseolina* and showed that seed infection due to *M. phaseolina* led to both pre and post emergence morality of okra confirming seed to seedling transmission of the pathogen.

Fakir (2000) listed 6 different fungal genera from okra seeds in Bangladesh namely *Aspergillus*, *Cercospora*, *Fusarium*, *Colletotrichum*, *Macrophomina* and *Penicillium*.

Prasad, *et al*. (2000) got twenty fungal species from stored okra seeds and the most dominant fungal species were *A. niger*, *A. flavus*, *A. sydowii*, *A. candidus*, *F. moniliforme* (*Gibberella fujikuroi*), *A. alteranata*, *Curvularia lunata* (*Cochliobolus lunatus*), *Penicillum oxaticum* and *Mucor* spp.

Rahman, *et al*. (2000) found that seed treatment with Vitavax showed higher shoot and root length which was followed by manually cleaned seed and floatation method.

Rashid (2000) listed six important pathogens of okra seeds in Bangladesh which were *Cercospora* spp, *Alternaria* spp, *Phyllostica* spp, *Colletotrichum dematium*, *Ascochyta abelmoschi*, *Choanephora cucurbitarum*, *Rhizoctonia* spp, *M. phaseolina*, *Fusarium* spp.

Quayam (1999) identified and reported l0 seed borne fungal pathogens in okra seeds from four different locations of Mymensingh Sadar. The predominant respect of prevalence were *Fusarium oxysporum*, *F. moniliforme*, *Macrophomina phaseolina*, *Aspergillus flavus* and *Colletotrichum dematium*.

Pun, *et al*. (1998) reported a total of 10 okra seed samples were associated with *M. phaseolina* and showed that infection led to both pre and post emergence mortality which confirmed the seed to seedling transmission of the pathogen.
Moretto, *et al*. (1997) reported that pathogenic fungi such as *Colletotrichum* spp, *Rhizoctonia solani, Fusarium* spp and *Alternaria* spp were associated with seeds of the okra.

Dash and Narain (1996) reported about the pre–treatment of seeds of most crops (okra, cowpea, sorghum, wheat, *Vigna radiata* and *luffa acutangula*) with Bavistin + TMTD (carbendazim + thiram) considerably improved seed germination. For most of the test crops, after Bavistin + TMTD, Thiram, Brassicol (quintozene), Difolatan (captafol), Dithane (Flowable) (mancozeb) and Vitavax (carboxin) eliminated seed borne mycoflora and improved seed germination.

Majid (1996) identified nine different fungi representing seven genera in okra seeds *viz- Aspergillus niger*, *Aspergillus* spp, *Alternaria* spp, *Colletotrichum dematium, Fusarium* spp, *Macrophomina phaseolina, Penicillium* spp and *Penicillium* spp detected as seed borne fungi.

Kasim (1996) isolated seed borne fungi of locally cultivated okra, capsicum, radish and soybean seeds and recorded the genera viz.- *Alternaria*, *Aspergillus*,

Botrytis, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Epicoccum*, *Fusarium*, *Penicillium* and *Stemphylum* etc. were isolated.

Al–Kassim (1996) reported a total of 15 species of fungi belonging to the genera *Alternaria*, *Aspergillus*, *Botrytis*, *Cladosporium*, *Colletotrichum*, *Curvularia*, *Epicoceum*, *Fusarium*, *Penicillum* and *Stemphylium* from okra seeds in Saudi Arabia. Carbendazim (as Bavistin), Benomyl (As Benlate), Copper oxychloride + zinc (as Cozib 62), Mancozeb (as Dithane M–45) and Metalaxyl (As Ridomil MZ–58) were used as seed treatments and the number of fungal species was greatly reduced when seeds were treated with the fungicides at concentration 0.2% before placing them on agar plates. Benomyl was the most efficient seed treatment, followed by Copper oxychloride + zinc and Mancozeb.

Fernandes, *et al*. (1992) recorded *Ascochyta abelmoschi*, *Botryodiplodia theobromae*, *Colletotrichum* spp, *Fusarium oxysporum*, *Macrophomina phaseolina*, *Phomopsis* spp, *Rhizoctonia solani*, *Aspergillus* spp, *Curvularia* spp, *Penicillum* spp, *Penicillium* spp, *Tricoderma* spp etc, isolated from okra seeds.

Ashrafuzzaman (1991) described that stem rot and wilt disease of okra were caused by *Macrophomina phaseolina* and *Fusarium oxysporum*, *F. vasinfectum* respectively. He also reported leaf spots of okra caused by a number of fungi, like species of *Alternaria*, *Cercospora* and *Phyllosticta*.

Richardson (1990) listed seven seed borne fungal pathogens on okra which were *Ascochyta abelmoschi*, *Choanephora curcubitirum*, *Colletotrichum dematium*, *Fusarium oxysporum*, *F. solani*, *Fusarium* spp and *Macrophomina phaseolina*.

2.3 Effect of containers for controlling the associated fungi of okra seed

Islam (2006) reported that the incidence of *Colletotrichum, Fusarium oxysporum* and *Aspergillus flavus* was minimum in tin container and was maximum in gunny bag in case of both neem and mehagoni extract.

2.4 Effect of seed treatment for controlling the associated fungi in okra seed

Nwangburuka, *et al*. (2012) studied the effects of *Moringa oleifera* leaf extract and Sodium hypochlorite seed pre-treatment on the seed germination, seedling growth rate and fungal activities. The results reveal that 4% and 6% of NaOCl inhibited the population of fungal growth; while Moringa extract reduced fungal growth and population. This explained that the pre-treatment of seeds before storage with Moringa reduced the possibility of fungal infection and also maintained the viability and vigor of the seed for a particular time period, depending on the seed type.

Mashooda and Lokesh (2011) found that to manage the fungal pathogens of okra seeds, seeds soaking with the aqueous leaf extracts of *Coleus aromaticus*, *Adathoda vesica*, *Vitex negundo*, *Solanum nigrum*, *Leucas aspera*, *Ocimum sanctum* and *Catharanthus roseus* were found superior in reducing the incidence of mycoflora.

Dawar, *et al*. (2008) found some root rot fungi *viz.*, *Macrophomina phaseolina*, *Rhizoctonia solani* and *Fusarium* spp on okra and sunflower plants. Of the different microbial antagonists used, *T. harzianum* was found more effective followed by *B. thuringiensis*, *R. meliloti* and *A. niger* in the control of root rot fungi.

Abduhu (2007) studied the efficacy of fungicides and botanicals to control major seed borne fungal pathogens of okra and six fungicides namely Vitavax-200, Dithane M-45, Rovral 50 WP, Ridomil MZ-68, Cupravit 50 WP and Bavistin 50 WP were used in the experiment. Among them Vitavax-200 was most effective to control *Aspergillus* spp, which was followed by Rovral-50 WP and Ridomil MZ-68. In controlling *Fusarium* spp, Ridomil MZ-68 was most effective, which was followed by Dithane M-45 and Rovral 50 WP.

Abduhu (2007) also experimented with five plants extract namely neem leaf, ginger rhizome, biskatali leaf, dholkalmi leaf and garlic clove and neem leaf extract was found to be the most effective in controlling *Aspergillus* spp, which was followed by dholkalmi and biskatali.

Tariq, *et al*. (2006) used the leaves, stem and pneumatophore of *Avicennia marina* in controlling the root infecting fungi viz., *Fusarium* spp, *Macrophomina phaseolina* and *Rhizoctonia solani* on mash bean and okra plants and found the infection of *Fusarium* spp, *R. solani* and *M. phaseolina* to be reduced significantly in okra and mash bean plants where soil amendment was used with *A. marina* plant parts powder @ 5% w/w which was effective in controlling root infecting fungi followed by stem and pneumatophore.

Karim (2006) studied fungal prevalence and their control with six seed treating agents viz. garlic clove extract, neem leaf extract, allamanda leaf extract, BAU–Biofungicide, Vitavax–200 and Bavistin 50 wp and among those, Vitavax–200 was found to be the most effective to control seed borne fungi.

Islam (2006) experimented with different treatments [T_0= control, T_1= 1:1 dilution, T_2= 1:2 dilution and T_3= 1:3 dilution of neem extract and mehagoni] and found the neem extract ie. T_2 were more effective for controlling *Colletotrichum dematium.*

Gurjar, *et al*. (2004) conducted an experiment to manage the seed borne pathogens of okra (*Curvularia* lunata, *Macrophomina phaseolina*,*Fusarium oxysporum*, *F. moniliforme* and *F. pallidorosum*) with bioagents (*Trichoderma harzianum, T. viride*, *Gliocladiium varens* and *Pseudomonas fluorescense*) and *T. viride* treatment recorded the highest germination against *F. pallidoroseum*, followed by *F. oxysoporum, M. phaseolina*, *F. moniliforme* and *C. lunata.*

Singh and Kumar (2003) conducted an experiment with okra to determine the efficacy of neem (*Azadirachta indica*) based pesticide against the fungal pathogen and found that the neem seed kernel extract at 1% was the most effective in controlling the insect of okra which was previously attacked by fungi.

Anam, *et al*. (2002) reported that the seed borne fungal diseases of okra *viz.* foot and root rot, anthracnose and die back, *Cercospora* leaf spot, *Corynespora* leaf spot and leaf blight, respectively caused by *Fusarium oxysporum, Colletotrichum dematium, Cercospora abelmoschi, Corynespora cassiicola* and *Macrophonina phaseolina* were found to be reduced by the use of seeds treated with Vitavax–200 and clean healthy seeds.

Ambekar, *et al*. (2000) also found that the neem products 0.5% achook was the best in reducing the incidence of fungi species as well as in reducing the fruit borer infestation.

Arun, *et al*. (1995) found that the extract of garlic bulb was effective in suppressing radial growth of the pathogens. On *Fusarium* and *Sclerotium* the extract was most effective when added after sterilization.

Jones, *et al*. (1992) reported that the *pseudomonas syringae* group was a very light group forming a cluster distinct from many of the fluorescent and all non–fluoprescent pseudomonas and from the other phyto–pathogenic bacteria.

CHAPTER III

MATERIALS AND METHODS

The details of the materials and methods of this research work were described in this chapter as well as on experimental site, experiment period, experimental materials and experimental design, data collection on seed quality as well as seedling attributes and seed borne fungi during storage. Overall discussion about experiment was carried out to study on the management of seed borne fungi and its impact on storage of okra seed and seed quality characters as well as seedling attributes under the following headings and sub-headings.

3.1 Experimental site

The quality observation of okra seed as moisture content and also prevalence of seed borne fungi and their infested percent of okra seed during storage at different months of storage were conducted in the Laboratory of the Department of Seed Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207.

3.2 Experimental period

The experiment was conducted during July 2013 to June 2014.

3.3 Atmospheric conditions of the storage room

The temperature and relative humidity of the storage room were recorded daily during the study period. The minimum and maximum temperatures during the study period of the storage room were 24.0 to 33.5^{o}C, respectively. The minimum and maximum relative humidity was 65 and 98%, respectively (Appendix VIII).

3.4 Seeds collection

The sample seeds of okra belong to the variety BARI Dherosh–1 was collected from the Horticulture Research Division, Bangladesh Agricultural Research Institute, Joydebpur, Gazipur, Bangladesh. The following photograph showing the sample seeds of BARI Dherosh–1 (Figure 1).

Fig. 1 : Collected okra seed (BARI Dherosh–1)

3.5 Experimental materials

3.5.1 Seeds of variety

Variety

The Okra variety BARI Dherosh–1 was used as experimental materials for the study which was released from Bangladesh Agricultural Research Institute (BARI).

3.5.2 Treatments

A. Storage container

The experiment comprised with the five containers treatments as storage packing materials. They are as follows (**Fig** 2):

T_1= Plastic container or pot (pc)

T_2= Plastic bag (pb)

T_3= Polythene coated gunny bag (pgb)

T_4= Gunny bag (gb)

T_5= Earthen pot (ep)

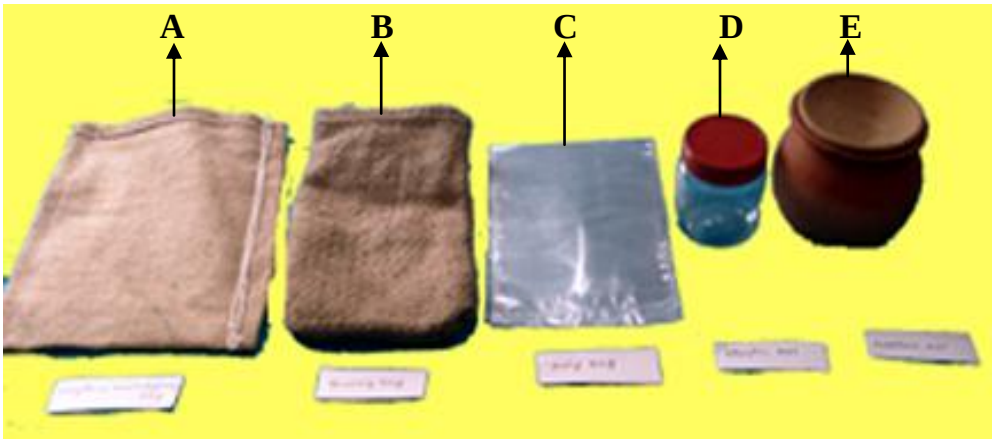


Fig. 2 : Photograph showing different container of storage; A) Polythene coated gunny bag (pgb), B) Gunny bag (gb), C) Plastic bag (pb), D) Plastic container or pot (pc) and E) Earthen pot (ep)

B. Seed treatment

The experiment consisted of the following three different seed treatment including control:

N_0= non treated seed (control)

N_1= Seed treated with provax-200

N_2= Seed treated with neem leaf powder

3.6 Experimental design and layout

The experiment consisted of two factors (five different packing materials as storage container were used as level factor A and the seed treatments were used as level factor B) and was laid out in Completely Randomized Design (CRD) with three replications. There were 45 (5 containers × 3 seed treatment × 3 replication) treatments were taken for the present study where 400 seeds were stored in each treatments. Thus there were 18000 (45 × 400) seeds were used in this experiment.

3.7 Preparation and application of post-harvest treatments

Four hundred seeds were randomly selected from there experimental seed lot for each treatment and placed on the table of the laboratory at ambient condition for the observation of seed quality and incidence of fungi in storage condition at different months of storage. Seedling attributes after germination of the selected storage seed of different months of storage were also evaluated in plant pathology laboratory.

Control treatment (okra seeds were not subjected to treatments) (N_0)

Freshly and matured harvested four hundred okra seeds were randomly selected from the experimental seed lot. Seeds were placed on the table in normal condition (room temperature) which was not related to any level of seed treatments but five different container treatments were used as packing materials.

Provax-200 (N_1)

The fungicide provax-200 acts as a broad spectrum were weighed accurately @ 2 g per kg of seed using electronic balance and dry seed treatment was done thoroughly in plastic container. A thorough mixing was done manually to have uniform dressing on all the seeds. The treated seeds were packed in polythene coated gunny bag, plastic pot, polybag, gunny bag and earthen pot.

Neem extracts (N_2)

Application technique : Freshly and matured harvested four hundred okra seeds were randomly selected from the experimental seed lot. The selected seeds were then dipped in neem powder extract in Petridis for 6 hours and then the plant extract was drained out from the Petridis. The treated seed were dried on blotting papers for 6 hours and then packed in different storage container as storage container treatments. Thereafter all the treated seeds packed in different containers were placed on the table of the laboratory at ambient condition for calculating the studied characters. However, the data on germination and seedling attributes were recorded.

Extract preparation : Initially 1 kg leaves were taken from the stock of neem leaf which was collected from the SAU campus and dried in sun for 3 to 4 days. Thereafter it was also dried in electric oven at $82^oC \pm 2^oC$ for 72 hrs. The dried leaves were crushing for making the powder. The prepared neem leaf powder were then dipped in 400 ml water using a blender through straining and then cheesed. The stock extract was then used to prepare treatment concentrations of 1:1 (400 g neem leaves powder: 400 ml water). The seeds were then dipped into the treatment solutions for 5 minutes to ensure that enough quantity of extract being absorbed. The treated seeds were allowed to air dry for a period of 10 min and then kept on storage container for observation.

3.8 Parameter study as quality tests

The following parameter were studied as quality of okra seeds

i. Percentage of moisture content
ii. Percentage of seed germination
iii. Shoot length of seedling
iv. Root length of seedling
v. Seedling vigor index (SVI)
vi. Dry weight of seedling
vii. Field emergence of seedling.

Besides, percentage of seed infection occurred by the different fungal species were also calculated during the period of study. Flowing six fungi species were recorded at different month of storage (4, 8 and 12 months of storage).

i. *Fusarium* spp
ii. *Chaetomium globosum*
iii. *Aspergilus flavus*
iv. *Aspergilus niger*
v. *Rhizopus stolonifer*
vi. *Curvularia* spp.

3.9 Method of studying characteristics

3.9.1. Moisture content

Moisture content of the seed samples determined prior to temporary storage by a digital electric moisture meter and the results were expressed in percentage on wet weight basis (ISTA, 2006). Two independent working samples of seeds were drawn from each sub–sample and ground in a grinder. Five grams of ground seeds of each working samples were dried in an oven at 130–133^{o}C for one hour. Percentage of moisture content was calculated using following formula.

$$\% \text{ Moisture content} = \frac{(M_2 - M_3)}{(M_2 - M_1)} \times 100$$

Where,

M_1 = Weight in grams of the container and its cover

M_2 = Weight in grams of the container, its cover and ground seed before drying and

M_3 = Weight in grams of the container, its cover and ground seed after drying

3.9.2 Germination test

Four hundred pure seeds 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 was 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.

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

Where,

X = Total number of seeds per paper towel

X_1 = Number of seedlings per paper towel

3.9.3 Shoot length of seedling

The same ten normal seedlings selected randomly for measurement of root length were used to record the shoot length. The shoot length was measured from the base of primary leaf to the base of hypocotyl and the mean shoot length was expressed in centimeters.

3.9.4 Root length of seedling

Final count was observed on 12th day and 10 normal seedlings were selected randomly and measured the root length of them. The root length was measured from the tip of primary root to base of the hypocotyl and the mean root length was expressed in centimeters.

3.9.5 Seedling vigor

For seedling vigor test, after 7 days of emergence 10 seedlings were randomly selected from each 400 seeds used for germination test. Altogether 40 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. Vigor index was computed following a standard formula as suggested by Abdul–Baki and Anderson (1973), where Vigor index = [Mean root length (cm) + mean shoot length (cm)] × germination (%). The following photograph showing the seedling on paper towel at 7 days after germination (Figure 3).

Fig. 3 : Seedlings germinated in paper towel method

3.9.6 Dry weight of seedling

Ten normal seedlings used for measuring the seedling length were put in the butter paper bag and dried in a hot air oven, maintained at 70 ± 1^{0}C temperature for 24 hours. Then the seedlings removed and allowed to cool in a desiccator for 30 minutes, the weighing was done in an electronic balance. The weight of dried samples recorded and average of ten seedling dry weight in grams recorded.

3.9.7 Field emergence of seedling

A sample of 400 seeds were drawn randomly from each of three replications in all the treatments and sown manually and covered with the fine soil. The spacing maintained between the seeds was 2.0 cm and between the rows was 15 cm; adequate soil moisture was maintained in the seed bed. The emergence count was taken on 15th day of sowing. The seedlings appearing on the surface of soil were considered as emerged. The field emergence values were expressed in percentage.

3.9.8 Seed health test

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

3.9.8.1 Prevalence of fungi associated with randomly selected okra seeds

A. Blotter method

A sub-sample of 200 seeds was randomly selected from each sample. Seeds were plated on sterilized and moist filter paper in 9 cm Petridis. Sixteen seeds were plated in each Petridis maintaining equal distances from seed to seed. Before planting, the filter paper was autoclaved at 121^0 C temperature and 1 kg/cm^2 pressure for 20 minutes. After planting the seeds were incubated at 25± 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 data 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 fungi 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.

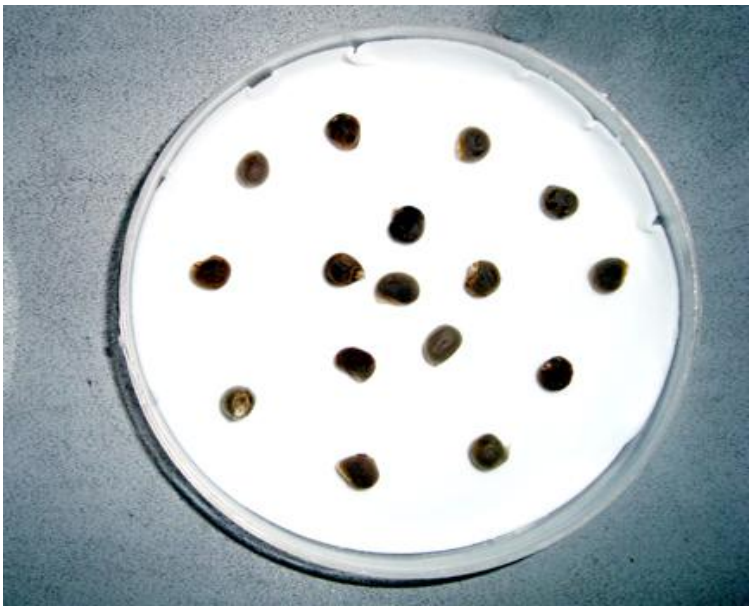


Fig. 4 : Seed health test by blotter method

B. Rolled paper towel method

Seedling vigor and seedling infection test was done in Rolled Paper Towel method (Rarham, 1990) In this method ,400 seeds randomly taken from each variety and 50 seeds were placed between a pair of moist paper towels in 10 × 5 direction .The towels were rolled and the two ends were closed with rubber band as the moist cloud not remove easily (Fig. 5) .Then the rolled papers containing seeds were placed in water contains large sized Petridis in an upright position in order to supply required moister 7 days at room temperature under normal 12/12 light and darkness cycle. After 10 days of incubation observation pertaining to (a) % germination (b) % diseased seedling (c) % dead seed (d) seedling weight (e) root length (f) shoot length (g) vigor index were recorded for determination of seedling vigor, 10 seedlings (normal/abnormal) were randomly selected from each paper and their individual root shoot was measured. Length of shoot was measured from the base of the stem up to the growing point of the youngest leaf. Similarly, length

of root was measured from starting point of the root to the largest available lateral root apex. Fresh weight of seedlings was taken before the materials could get desiccated. Vigor of the seedling was determined by the following formula (Baki and Anderson, 1972).

Vigor Index = (Mean of root length + Mean of shoot length) x % of seed Germination.

Fig. 5 : Rolled paper towel method

3.9.8.2 Pathogenicity test

A. Preparation of soil

The soil was prepared by sterilized (121^0 C temp., psi 15 min) in an autoclave (Fig. 6).

B. Raising of seedlings

Seeds were sown at a constant rate on sterilized soil. Time to time observation and watering was done daily and whenever necessary .After 12-15 days data was recorded. (Fig. 7).

Fig. 6 : Pathogen city test

Fig. 7 : Raising of seedlings

3.10 Analysis of data

The data of all tests were analyzed statistically for analysis of variance (ANOVA) using MSTAT-C computer program. The means were adjusted by Duncan's Multiple Range Test (DMRT) and Least Significant Difference (LSD) at both 0.5% level of significance using same computer program. Whenever necessary the data were transformed before statistical analysis following appropriate method.

CHAPTER IV

RESULTS

4.1 Evaluation of quality attributes of okra seed as influence by storage containers and seed treatments

4.1.1 Moisture content (%) of okra seed

Effect of storage containers

Moisture content of seeds sample during storage varied significantly from 8.03 to 16.40% for 4 months of storage, 8.21 to 16.44% for 8 months of storage and 8.56 to 17.63% for 12 months of storage due to various containers and coefficient of variation was 1.00% (Appendix I and Fig. 8). It is evident from the Fig. 8 that the moisture content was lowest in those seeds stored in plastic container (8.56%) followed by plastic bag (8.64%) at 12 months of storage and statistically differed from other treatments at 4 and 8 months of storage. On the other hand, moisture content was maximum in gunny bag which also statistically differed from other treatments at all the storage period.

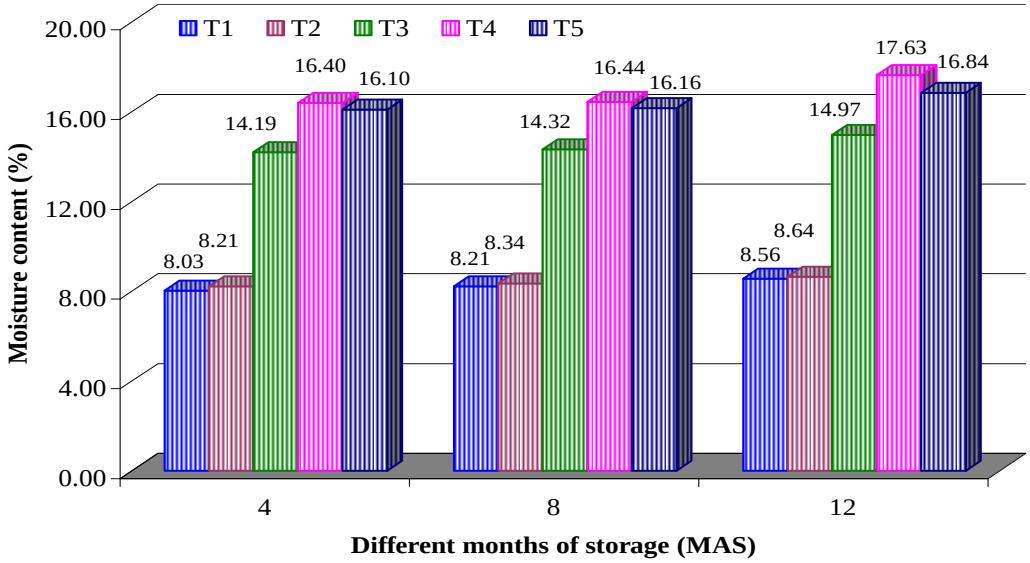
Effect of seed treatments

Analysis of variation data on moisture content differed significantly due to seed treatment chemicals in the storage period (Appendix I and Fig. 9). Among the seed treatment, in provax-200 recorded lower moisture content at 4, 8 and 12 months after storage (12.52%, 12.65%, 13.27%) followed by neem leaf powder (12.61%, 12.71%13. 33%). In non-treated seed maximum values of moisture respectively was recorded which was much higher than the standard moisture level of okra (Anon, 2006).

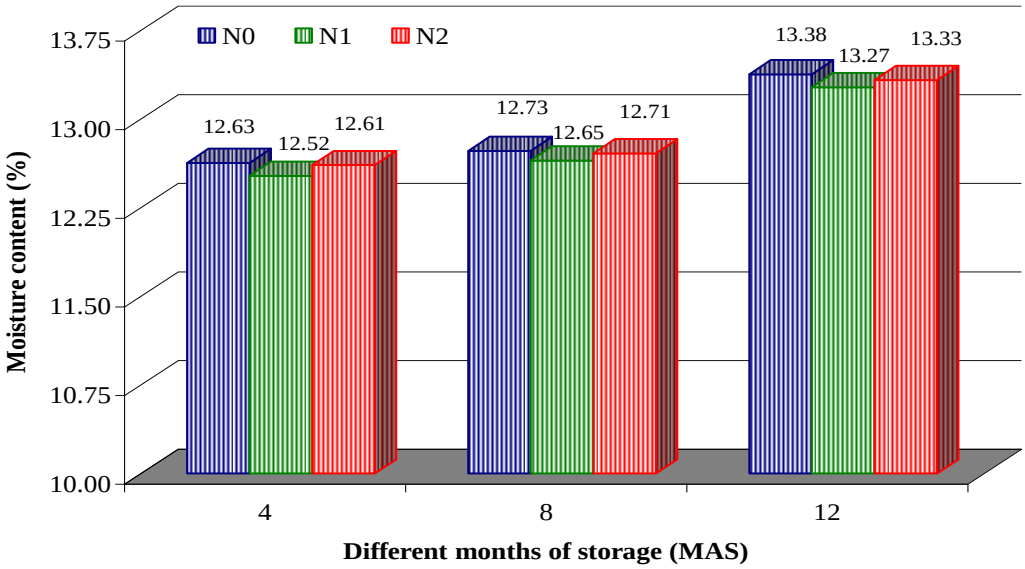
Combined effect of storage containers and seed treatments

Among the combined treatments, moisture content had minimum in those seeds which were treated by provax-200 and stored in plastic container (7.98%, 8.16%,

8.48% at 4, 8 and 12 MAS) followed by all the treated seeds stored in both plastic container and plastic bag ,obtained the statistically identical minimum moisture content at 8 month (8.21%, 8.27% and 8.37%, 8.32%).



T1: Plastic container; T2: Plastic bag; T3: Polythene coated gunny bag; T4: Gunny bag and T5: Earthen pot
Fig. 8 : Effect of different storage containers on percentage of moisture content of storage okra seed at different months of storage



N_0: Control (Non - treated); N_1: Seed treated with provax-200 and N_2 : (NLP) Seed treated with neem leaf powder extract

Fig. 9 : Effect of seed treatments on percentage of moisture content of storage okra seed at different months of storage.

4.1.2 Germination (%) of okra seed

Effect of storage containers

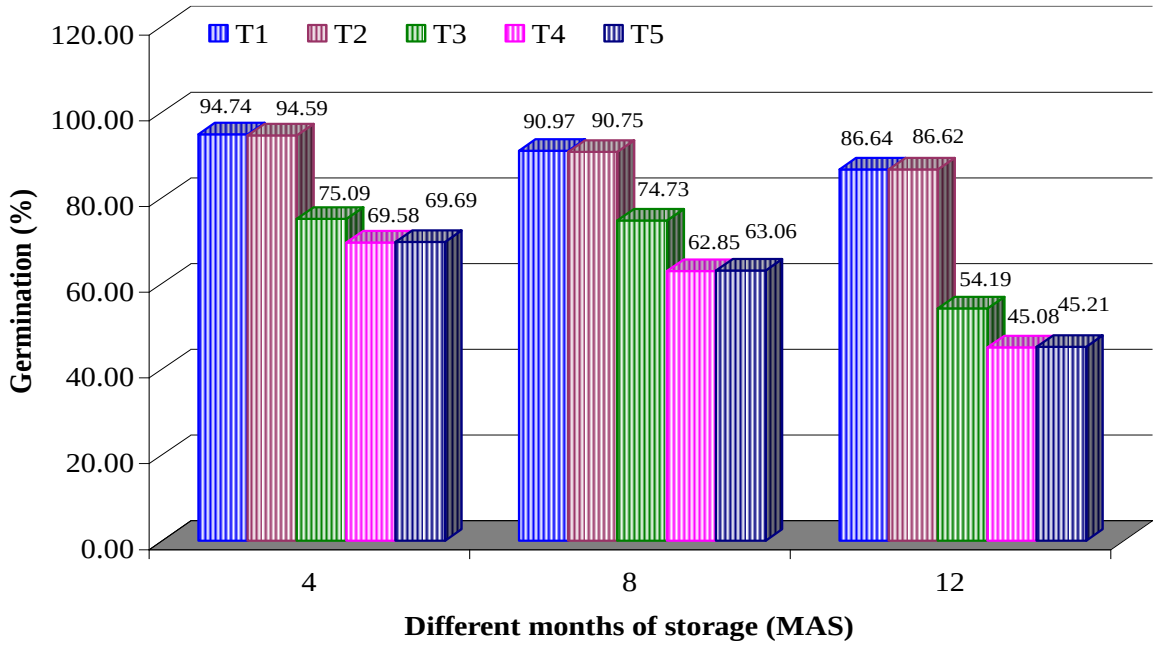
A significant variation was found in respect of seed germination at 4, 8 and 12 months of storage where germination percentage of those storage seed varied from 69.58 to 94.74% at 4 months of storage, 62.85 to 90.97% at 8 months of storage and 45.08 to 86.64% at 12 months of storage (Appendix I and Fig. 10). Among the various treatments on storage container, germination percentage had maximum in those seeds stored in plastic container (94.75%, 90.97% and 86.64%, respectively) at those 4, 8 and 12 months of storage followed by plastic bag(94.59, 90.75, and 86.62%). On the other hand, seed stored in gunny bag produced significantly lowest germination while it did not differed significantly with those seed stored in earthen pot (69.69, 63.06 and 45.21%) at 4, 8 and 12 months of storage, respectively.

Effect of seed treatments

Germination percentage of the stored seeds at 4, 8 and 12 months were influenced significantly due to various seed treatments (Appendix I and Fig.11). Among the seed treatments, in provax-200 recorded maximum germination percentage (82.92, 78.75 and 66.37% respectively) followed by the treated seeds of neem leaf powder (79.98, 76.14 and 64.10%) and non- treated seed (79.31, 74.53 and 60.17%) at 4, 8 and 12 months after storage, respectively where untreated and neem leaf treated seeds were statistically similar at 4 months of storage (Fig. 11).

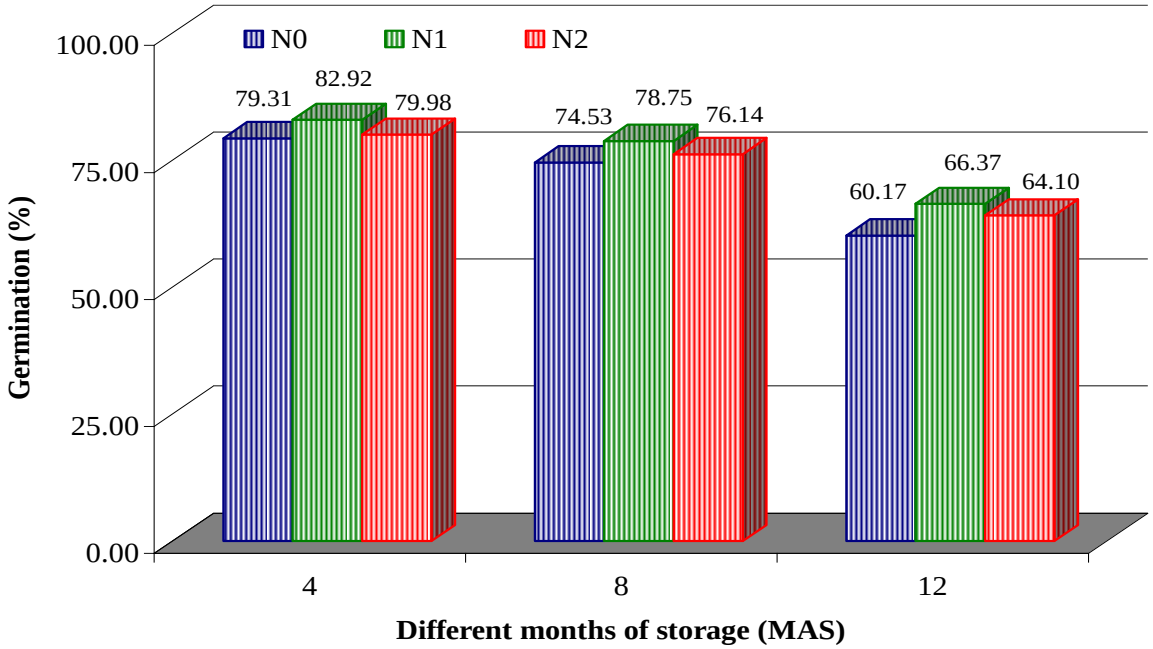
Combined effect of storage containers and seed treatments

Germination percentage due to combine effect between storage containers and seed treatments was highly significant at 4, 8 and 12 months of storage (Appendix I and Table 1). The treated seed of provax-200 stored in plastic container produced significantly highest percentage of germination (96.74%, 93.74% and 88.84% respectively) at 4, 8 and 12 months of storage followed by similar treated seed stored in plastic bag (96.55, 93.75 and 88.75%, respectively).



T1: Plastic container; T2: Plastic bag; T3: Polythene coated gunny bag; T4: Gunny bag and T5: Earthen pot

Fig. 10 : Effect of different storage containers on percentage of seed germination of okra seed at different months of storage



N_0: Control (Non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

Fig. 11 : Effect of seed treatments on percentage of seed germination of okra seed at different months of storage

Table 1 : Interaction effect of different storage containers and seed treatments on percentage of moisture content and germination of okra from the seed of different months of storage

Treatments	Moisture content (%) at different months of storage			Germination (%) at different months of storage		
	4	8	12	4	8	12
$T_1 \times N_0$	8.02 fg	8.21 d	8.65 d	93.56 b	89.46 b	85.16 b
$T_1 \times N_1$	7.98 g	8.16 d	8.48 d	96.74 a	93.74 a	88.84 a
$T_1 \times N_2$	8.09 efg	8.27 d	8.54 d	93.92 b	89.72 b	85.92 b
$T_2 \times N_0$	8.27 e	8.37 d	8.70 d	93.47 b	88.77 b	84.77 b
$T_2 \times N_1$	8.12 efg	8.32 d	8.59 d	96.55 a	93.75 a	88.75 a
$T_2 \times N_2$	8.23 ef	8.33 d	8.63 d	93.74 b	89.74 b	86.34 b
$T_3 \times N_0$	14.25 d	14.35 c	15.00 c	72.75 de	73.75 d	51.07 e
$T_3 \times N_1$	14.14 d	14.29 c	14.94 c	77.77 c	76.73 c	56.77 c
$T_3 \times N_2$	14.19 d	14.33 c	14.98 c	74.74 d	73.71 d	54.73 d
$T_4 \times N_0$	16.44 a	16.48 a	17.67 a	68.23 f	60.03 f	39.71 h
$T_4 \times N_1$	16.35 ab	16.39 a	17.57 a	71.76 e	64.77 e	48.77 f
$T_4 \times N_2$	16.42 a	16.45 a	17.66 a	68.76 f	63.76 e	46.76 g
$T_5 \times N_0$	16.15 bc	16.25 ab	16.89 b	68.55 f	60.64 f	40.15 h
$T_5 \times N_1$	16.03 c	16.08 b	16.78 b	71.76 e	64.76 e	48.74 f
$T_5 \times N_2$	16.12 c	16.16 b	16.86 b	68.76 f	63.77 e	46.75 g
$LSD_{(005)}$	**0.2109**	**0.2174**	**0.2358**	**2.112**	**2.022**	**1.617**
CV(5%)	**1.00**	**1.02**	**1.05**	**1.57**	**1.59**	**1.53**

T1: Plastic container; T2: Plastic bag; T3: Polythene coated gunny bag; T4: Gunny bag and T5: Earthen pot

N_0: Control (Non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

4.1.3 Shoot length of seedlings

Effect of storage containers

The length of shoot of seedlings was longest (20.76 cm, 17.87 cm and 14.97 cm respectively) showed in those seeds which were stored in plastic container after 4, 8 and 12 months of storage followed by plastic bag (20.69 cm, 17.59 cm,14.89 cm respectively). On the other hand, shoot length of the germinated seedlings from the seed stored at 4 and 12 months of storage in gunny bag showed the shortest shoot (15.15 and 11.49 cm, respectively) which statistically differed from other storage container treatments (Appendix I and Table 2).

Effect of seed treatments

Analysis of variance data regarding shoot length of seedling were statistically significant due to the studied seed treatments where seed treated with provax-200 produced longest shoot (17.74, 14.58 and 13.18 cm) followed by seed treated with neem leaf powder extract (17.57, 14.46 and 12.98 cm). On the other hand non-treated seed produced shortest shoot (17.35, 14.29 and 12.77 cm) from the storage seeds of 4, 8 and 12 months (Appendix II and Table 3).

Combined effect of storage containers and seed treatments

A significant variation due to combined treatments between storage containers and seed treatments regarding shoot length of 4, 8 and 12 months of storage seeds of okra in this study where the longest shoot of okra seedlings were obtained from the provax-200 treated seed stored in plastic container (21.04 cm, 18.14 cm, 15.24 cm respectively) at 4, 8 and 12 months of storage followed by neem leaf powder extract treated seeds stored in plastic container (20.79, 17.92 and 15.02 cm respectively). Non-treated seeds stored in gunny bag produced significantly the shortest shoot (15.03, 12.13 and 11.33 cm) which was statistically from other combine treatments at 4, 8 and 12 months of storage (Appendix II and Table 4).

4.1.4 Root length of seedlings

Effect of storage containers

The longest and statistically identical root length of seedlings was observed with the treatments in plastic containers (11.52, 9.830 and 8.507 cm respectively) followed by plastic bag (11.42, 9.637 and 8.11 cm respectively) at 4, 8 and 12 months after storage of okra seeds (Appendix II and Table 2). Lower significant response in length of root (8.36, 6.49 and 6.34 cm) was found when storage container was gunny bag. And also did not differed significantly with earthen pot and polythene coated gunny bag at 8 months of storage (6.54 and 6.59 cm, respectively) and 12 months of storage (6.36 and 6.52 cm, respectively).

Table 2. Effect of different storage containers on length of shoot and root of okra seedlings from the seed of different months of storage

Treatments	Shoot length of seedling at different months of storage			Root length of seedling at different months of storage		
	4	8	12	4	8	12
T_1 (pc)	20.76 a	17.87	14.97 a	11.52 a	9.830 a	8.507 a
T_2 (pb)	20.69 a	17.59	14.89 a	11.42 a	9.637 b	8.113 a
T_3 (pgb)	15.74 b	12.28	11.84 b	8.683 b	6.590 c	6.520 b
T_4 (gb)	15.15 d	12.19	11.49 c	8.360 c	6.487 c	6.343 b
T_5 (ep)	15.43 c	12.3	11.69 b	8.527 bc	6.540 c	6.363 b
$LSD_{(005)}$	**0.2455**	**0.1552**	**0.1552**	**0.1877**	**0.146**	**0.7432**
CV (%)	**1.45**	**1.12**	**1.25**	**2.01**	**1.92**	**10.77**

Pc: Plastic container (T1); Pb: Plastic bag (T2); Pgb: Polythene coated gunny bag (T3); Gb: Gunny bag (T4) and Ep: Earthen pot (T5)

Effect of seed treatments

Analysis of variance regarding root length of seedlings from the storage seeds of 4 and 8 months showed significant difference due to seed treatment while root length did not vary significant at the seedlings of 12 months after storage seed (Appendix II and Table 3). From the seed treatments, seed treated with provax-200

produced significantly the longest root (9.94, 8.048 and 7.412 cm respectively) followed by seed treated with neem leaf powder (9.65 and 7.740 and 7.104 cm) after 4, 8 and 12 months of storage.

Combined effect of storage containers and seed treatments

A significant variation due to combined treatments between storage containers and seed treatments regarding shoot length of seeds 4, 8 and 12 months after storage seeds of okra in this study where the longest root of okra seedlings were obtained from the provax-200 treated seed stored in plastic container (11.91 cm, 10.25 cm, 8.900 cm respectively) at 4, 8 and 12 months of storage followed by neem leaf powder extract treated seeds stored in plastic container (11.38 cm, 9.680 cm and 8.360 cm respectively). Non-treated seeds stored in gunny bag produced significantly the shortest roots which was statistically from other combine treatments at 4 and 12 months of storage (Appendix II and Table 3)

Table 3. Effect of seed treatments on length of shoot and root of okra seedling from the seed of different storage periods.

Treatments	Shoot length of seedling at different months of storage			Root length of seedling at different months of storage		
	4	8	12	4	8	12
N_0 (NT)	17.35b	14.29 b	12.77 c	9.508 c	7.662 b	6.992
N_1 (Pvx)	17.74 a	14.58 a	13.18 a	9.940 a	8.048 a	7.412
N_2 (NLP)	17.57 a	14.46 a	12.98 b	9.654 b	7.740 b	7.104
$LSD_{(005)}$	**0.1901**	**0.1202**	**0.1131**	**0.1454**	**0.1131**	**0.5757**
CV (%)	**1.45**	**1.12**	**1.25**	**2.01**	**1.92**	**10.77**

N_0: Control (Non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

Table 4. Interaction effect of different storage containers and seed treatments on length of shoot and root of okra seedling from the seed of different of storage periods.

Treatments	Shoot length of seedling at different months of storage			Root length of seedling at different months of storage		
	4	**8**	**12**	**4**	**8**	**12**
$T_1 \times N_0$	20.46 b	17.56 c	14.66 c	11.26 b	9.560 bc	8.260 a
$T_1 \times N_1$	21.04 a	18.14 a	15.24 a	11.91 a	10.25 a	8.900 a
$T_1 \times N_2$	20.79 ab	17.92 ab	15.02 ab	11.38 b	9.680 b	8.360 a
$T_2 \times N_0$	20.37 b	17.27 d	14.57 c	11.16 b	9.370 c	7.870 abc
$T_2 \times N_1$	20.95 a	17.85 b	15.15 ab	11.81 a	10.05 a	8.500 a
$T_2 \times N_2$	20.74 ab	17.64 bc	14.94 b	11.28 b	9.490 bc	7.970 ab
$T_3 \times N_0$	15.65 cd	12.25 e	11.75 defg	8.540 cde	6.530 de	6.380 d
$T_3 \times N_1$	15.87 c	12.33 e	11.97 d	8.840 c	6.700 d	6.670 bcd
$T_3 \times N_2$	15.70 cd	12.26 e	11.80 def	8.670 cd	6.540 de	6.510 cd
$T_4 \times N_0$	15.03 f	12.13 e	11.33 h	8.230 e	6.360 e	6.200 d
$T_4 \times N_1$	15.27 def	12.25 e	11.67 efg	8.480 cde	6.670 d	6.520 cd
$T_4 \times N_2$	15.16 ef	12.19 e	11.46 gh	8.370 de	6.430 de	6.310 d
$T_5 \times N_0$	15.25 def	12.25 e	11.55 fgh	8.350 de	6.490 de	6.250 d
$T_5 \times N_1$	15.56 cde	12.33 e	11.86 de	8.660 cd	6.570 de	6.470 cd
$T_5 \times N_2$	15.47 cdef	12.31 e	11.67 efg	8.570 cde	6.560 de	6.370 d
$LSD_{(005)}$	**0.4251**	**0.2689**	**0.2689**	**0.3251**	**0.2529**	**1.287**
CV (%)	**1.45**	**1.12**	**1.25**	**2.01**	**1.92**	**10.77**

T1: Plastic container; T2: Plastic bag; T3: Polythene coated gunny bag; T4: Gunny bag and T5: Earthen pot

N_0: Control (Non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

4.1.5 Seedling vigor index (SVI)

Effect of storage containers

The data on SVI were recorded at 12 days after germination from the storage seed of 4, 8 and 12 months of storage where significantly the longest SVI was registered from the treatments plastic containers having 3087.00, 2425.0 and 2173.0 at 4, 8 and 12 months of storage followed by plastic bag respectively. On the other hand, statistically lower and identical SVI were obtained with the treatments gunny bag (1492.0, 1261.0 and 838.10 respectively) at 4, 8 and 12 months of storage (Table 5).

Effect of seed treatments

Analysis of variance data on root length of seedlings was significantly influenced due to see seed treatments where the treated seed of provax-200 registered the highest SVI (2300.0, 1831.0 and 1494.0) followed by neem leaf powder extract treated seed (2136.0, 1728.0 and 1401.0). And the lowest SVI observed non-treated seed (2123.0, 1686.0 and 1313.0), those seed which was collected from the storage seeds of 4, 8 and 12 months after storage (Appendix II and Table 6).

Table 5. Effect of different storage containers on seedling vigor index and dry weight of seedling from the seed of different storage periods.

Treatments	Seedling vigor index at different months of storage			Dry weight of seedling at different months of storage		
	4	8	12	4	8	12
T_1 (pc)	3087.0 a	2425.0 a	2173.0 a	55.60 a	48.97 a	42.56 a
T_2 (pb)	2972.0 b	2384.0 b	2119.0 b	55.59 a	48.73 a	42.51 a
T_3 (pgb)	1859.0 c	1394.0 c	1030.0 c	45.56 b	34.57 b	33.39 b
T_4 (gb)	1492.0 d	1261.0 d	838.10 d	44.77 b	33.71 b	33.40 b
T_5 (ep)	1523.0 d	1278.0 d	851.50 d	45.18 b	34.40 b	33.66 b
$LSD_{(005)}$	**67.09**	**35.74**	**28.45**	**2.096**	**3.38**	**2.762**
CV (%)	**3.19**	**2.13**	**2.11**	**4.41**	**8.76**	**7.73**

Pc: Plastic container (T1); Pb: Plastic bag (T2); Pgb: Polythene coated gunny bag (T3); Gb: Gunny bag (T4) and Ep: Earthen pot (T5)

Table 6. Effect of seed treatments on seedlings vigor index and dry weight of seedlings from the seed of different storage periods.

Treatments	Seedling vigor index at different months of storage			Dry weight of seedling at different months of storage		
	4	**8**	**12**	**4**	**8**	**12**
N_0 (NT)	2123.0b	1686.0 c	1313.0 c	49.29	39.83	37.06
N_1 (Pvx)	2300.0a	1831.0 a	1494.0 a	49.40	40.3	37.14
N_2 (NLP)	2136.0b	1728.0 b	1401.0 b	49.34	40.1	37.11
$LSD_{(005)}$	**51.97**	**27.76**	**22.04**	**1.623**	**2.618**	**2.139**
CV (%)	**3.19**	**2.13**	**2.11**	**4.41**	**8.76**	**7.73**

N_0: Control (Non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

Combined effect of storage containers and seed treatments

The data concerning SVI among the whole combine treatments varied significantly with an average range from 1411.0 to 3229.0 at 4 months of storage, 1223.0 to 2549.0 at 8 months of storage and 731.30 to 2300.0 at 12 months of storage (Appendix II and Table 7). Among the combine treatments, SVI had highest in T_1N_1 (plastic container × seed treated with provax-200) at the whole storage period (4, 8 and 12 months of storage) followed by T_2N_1 (plastic bag × seed treated with provax-200) at 4, 8 and 12 months of storage (3197.0, 2501.0 and 2253.0, respectively). Correspondingly, T_4N_0 (gunny bag × non-treated seed) produces the lowest SVI while statistically identical lowest SVI was also obtained by other treatment combination.

Table 7. Interaction effect of different storage containers and seed treatments on seedling vigor index and dry weight of seedlings from the seed of different storage periods.

Treatments	Seedling vigor index at different months of storage			Dry weight of seedling at different months of storage		
	4	8	12	4	8	12
$T_1 \times N_0$	2997.0 b	2328.0 cd	2081.0 c	55.54 a	48.68 a	42.54 a
$T_1 \times N_1$	3229.0 a	2549.0 a	2300.0 a	55.72 a	49.60 a	42.58 a
$T_1 \times N_2$	3034.0 b	2397.0 b	2137.0 b	55.58 a	48.63 a	42.55 a
$T_2 \times N_0$	2972.0 b	2280.0 d	2019.0 d	55.55 a	48.39 a	42.50 a
$T_2 \times N_1$	3197.0 a	2501.0 a	2253.0 a	55.63 a	49.37 a	42.53 a
$T_2 \times N_2$	2747.0 c	2372.0 bc	2086.0 c	55.59 a	48.42 a	42.50 a
$T_3 \times N_0$	1793.0 e	1358.0 f	979.60 f	45.52 b	34.16 b	33.35 b
$T_3 \times N_1$	1941.0 d	1451.0 e	1078.0 e	45.59 b	34.29 b	33.43 b
$T_3 \times N_2$	1843.0 de	1372.0 f	1032.0 e	45.56 b	35.26 b	33.39 b
$T_4 \times N_0$	1411.0 h	1223.0 h	731.30 i	44.71 b	33.61 b	33.33 b
$T_4 \times N_1$	1551.0 fg	1319.0 fg	913.10 gh	44.81 b	33.78 b	33.45 b
$T_4 \times N_2$	1514.0 fgh	1240.0 h	870.00 h	44.78 b	33.74 b	33.41 b
$T_5 \times N_0$	1441.0 gh	1242.0 h	753.00 i	45.12 b	34.30 b	33.60 b
$T_5 \times N_1$	1583.0 f	1335.0 f	924.00 g	45.21 b	34.45 b	33.69 b
$T_5 \times N_2$	1544.0 fg	1258.0 gh	877.50 gh	45.21 b	34.44 b	33.68 b
$LSD_{(005)}$	**116.2**	**62.07**	**49.27**	**2.63**	**5.855**	**4.783**
CV (%)	**3.19**	**2.13**	**2.11**	**4.41**	**8.76**	**7.73**

T_1: Plastic container; T_2: Plastic bag; T_2: Polythene coated gunny bag; T_4: Gunny bag and T_5: Earthen pot

N_0: Control (Non-treated); N_1: Seed treated with provax and N_2: (NLP) Seed treated with neem leaf powder extract

4.1.6 Dry weight of seedling (g)

Effect of storage containers

There was significant variation due to storage container treatments in respect of dry weight of seedlings at all the data recording period whereas. Data on dry weight of seedlings was recorded at 12 days after germination (Appendix III and Table 5). The highest weight of dry seedlings was obtained in plastic container (55.60, 48.97 and42.56 g respectively) followed by plastic bag (55.59, 48.73 and 42.51 g respectively) at 4, 8 and 12 months after storage. Rest of the treatments

showed the lowest and statistically similar weight of dry seedling at 4, 8 and 12 months of storage where gunny bag was the much lower than other containers.

Effect of seed treatments

Analysis of variance data on root length of seedlings was significantly influenced due to seed treatments where the treated seed of provax-200 registered the highest dry weight of seedlings (49.40, 40.3 and 47.14 g respectively) followed by neem leaf powder extract treated seed (49.34, 40.1 and 37.11 respectively). The lowest dry weight of seedlings observed non- treated seed (49.29, 39.83 and 37.06, respectively), those seeds which was collected from the storage seeds of 4, 8 and 12 months after storage (Appendix II and Table 6).

Combined effect of storage containers and seed treatments

The data regarding dry weight of seedlings due to combine treatments between storage containers and seed treatments did not vary significantly as well as all the treatments of combination were statistically identical with each other (Appendix II and Table 7).). Among the combined effect of treatments, the dry weight of seedlings (55.72, 49.60 and42.58 g respectively) had highest in T_1N_1 (plastic container × seed treated with provax-200) at the storage period of 4, 8 and 12 months of storage followed by T_2N_1 (plastic bag × seed treated with provax-200) at 4, 8 and 12 months (55.63, 49.37 and 42.53, respectively).

However, it was significantly varied from 44.71 (T_4N_0) to 55.72 (T_1N_1) at 4 months of storage, 33.61 (T_4N_0) to 49.60 (T_1N_1) at 8 months of storage and 33.33 (T_4N_0) to 42.58 (T_1N_1) at 12 months of storage.

4.1.7 Field emergence

Effect of storage containers

The data on field emergence of seedlings was significantly influenced by the effect of storage container treatments at all the storage period of okra seed (4, 8 and 12 months of storage) where it was varied from 63.56 to 88.78% at 4 months of

storage, 57.06 to 85.25% at 8 months of storage and 37.36 to 80.21% at 12 months of storage (Appendix IV and Fig. 12). It was appeared that the maximum and statistically identical field emergence of seedlings was observed with the treatments plastic containers (88.78, 85.25 and 80.28% respectively) followed by plastic bag (87.87, 84.67 and 79.76%) after 4, 8 and 12 months of storage. Treatment T_4 had much lower than that of other treatments. The above result showed also the filled emergence of gunny bag minimum under same condition.

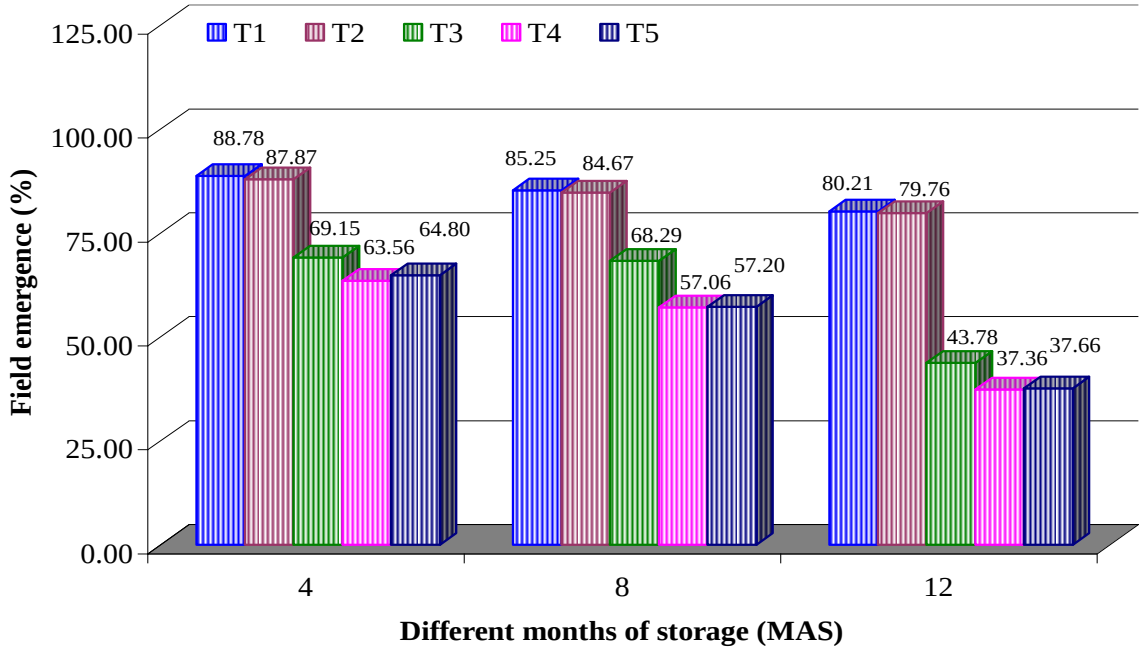
Effect of seed treatments

A significant variation regarding field emergence of seedling was also found due to seed treatments at 4, 8 and 12 months of storage seed (Appendix IV and Fig. 13). Among the seed treatments, the field emergence of seedling had highest (76.79, 72.65 and 58.53%) in those seeds which were treated by provax-200 (N_1) followed by the treated seeds of neem leaf powder extract (75.58, 70.95 and 55.23%) and low field emergence were founded without treated seed (73.13, 67.89 and 53.51%) at 4, 8 and 12 months of storage, respectively .

Combined effect of storage containers and seed treatments

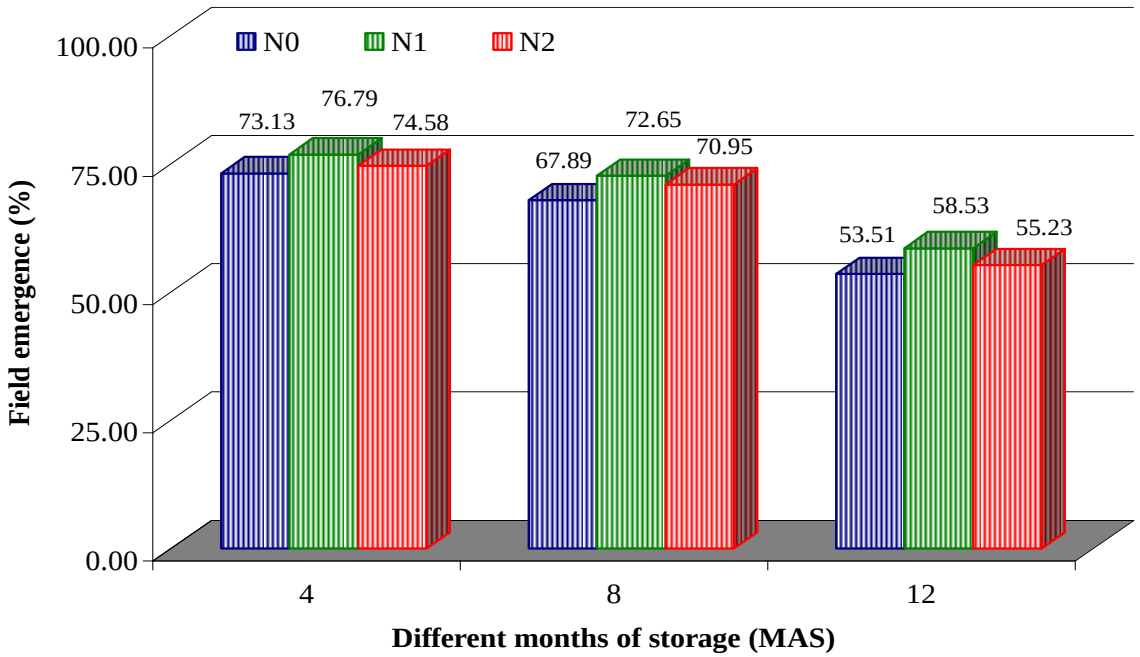
Field emergence of seedlings had also significant due to combine effect between storage container treatments and seed treatments where it was significantly varied from 61.51 to 90.66% at 4 months of storage, 54.51 to 88.09% at 8 months of storage and 34.51 to 81.53% at 12 months of storage (Appendix IV and Table 8).

As a result, field emergence (90.66, 88.09 and 81.53%) had maximum in T_1N_1 (plastic container × seed treated with provax-200) followed by T_2N_1 (plastic bag x seed treated with provax-200). Among other interaction treatments, treatment T_4N_0 (gunny bag × non- treated seed) obtained the lowest field emergence of seedlings where statistically similar minimum field emergence was produced by the combine treatments of T_4N_2, T_5N_0 at 4 months of storage, T_4N_1, T_4N_2, T_5N_0, T_5N_1 and T_5N_2 at 8 months of storage (57.85, 58.83, 52.92, 58.84 and 59.85%, respectively) and T_5N_0 at 12 months of storage (34.52%).



Pc: Plastic container; Pb: Plastic bag; Pgb: Polythene coated gunny bag; Gb: Gunny bag and Ep: Earthen pot

Fig.12. Effect of different storage containers on field emergence of seedling (%) of okra at different months of storage



N_0: Control (non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

Fig. 6. Effect of seed treatments on field emergence of seedling (%) of okra at different months of storage periods

Table 8. Interaction effect of different storage containers and seed treatments on field emergence of seedling from the seed of different storage periods.

Treatments	Field emergence at different months of storage		
	4	8	12
$T_1 \times N_0$	87.84 a	83.04 a	79.14 a
$T_1 \times N_1$	90.66 a	88.09 a	81.53 a
$T_1 \times N_2$	87.85 a	84.63 a	79.97 a
$T_2 \times N_0$	86.65 a	82.55 a	78.55 a
$T_2 \times N_1$	89.94 a	87.84 a	81.65 a
$T_2 \times N_2$	87.03 a	83.63 a	79.09 a
$T_3 \times N_0$	67.52 bc	66.42 b	40.82 cd
$T_3 \times N_1$	71.64 b	70.64 b	48.00 b
$T_3 \times N_2$	68.30 bc	67.82 b	42.52 c
$T_4 \times N_0$	61.51 c	54.51 c	34.51 e
$T_4 \times N_1$	65.65 bc	57.85 c	40.55 cd
$T_4 \times N_2$	63.53 c	58.83 c	37.03 de
$T_5 \times N_0$	62.12 c	52.92 c	34.52 e
$T_5 \times N_1$	66.08 bc	58.84 c	40.90 cd
$T_5 \times N_2$	66.19 bc	59.85 c	37.55 de
LSD (005)	**6.821**	**6.442**	**3.676**
CV(%)	**5.47**	**5.48**	**3.95**

Pc: Plastic container; Pb: Plastic bag; Pgb: Polythene coated gunny bag; Gb: Gunny bag and Ep: Earthen pot

N_0: Control (Non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

4.2 On Prevalence of seed borne fungi on okra seed during different storage periods Influence of storage containers and seed treatments

There are six seed borne fungi found associated with the seeds of okra during the whole storage period. The fungi associated with the seeds were *Fusarium* spp, *Colletotrichum* spp, *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer* and

Curvularia spp. Initially the total seed borne infection was recorded in collected okra seed the initial fungal prevalence were *Fusarium* spp 3.57%, *Chaetomium globosum* 3.52%, *Aspergillus flavus* 4.83%, *Aspergillus niger* 4.37%, *Rhizopus stonifer* 3.17% and *Curlvularia* spp 2.90%. Thereafter these seed borne fungi significantly increase and recorded at 4, 8 and 12 months after storage which were presented below with the some headings and sub headings.

4.2.1 Prevalence of *Fusarium* spp

Effect of storage containers

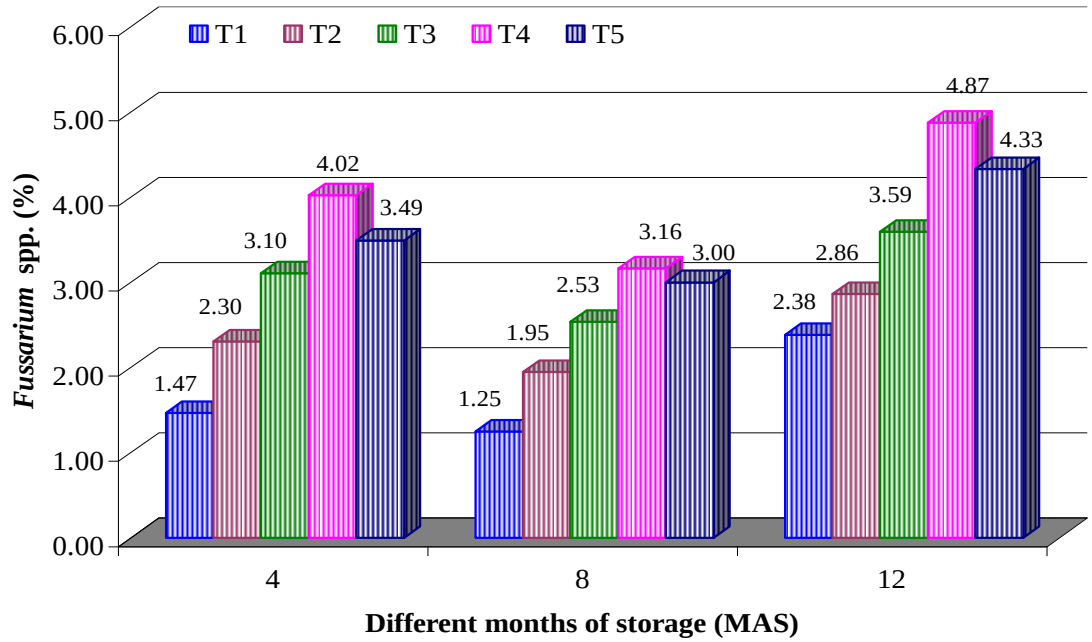
The difference of prevalence of *Fusarium* spp was statistically significant among the storage containers at all the storage period (Appendix V and Fig. 14 and Fig. 16-17). Prevalence of *Fusarium* spp (4.02 and 4.87%, respectively) was found highest in those seeds which were stored in gunny bag at 4 and 12 months of storage due to high moisture, followed by earthen pot (3.49 and 4.37%, respectively) at those storage period.

Effect of seed treatments

Effect of seed treatments on the prevalence of *Fusarium* spp during the storage period (4, 8 and 12 months of storage) had highly significant difference (Appendix V and Fig. 15). This study showed that non treated seed was highly affected by *Fusarium* spp due to more moisture, so maximum prevalence was obtained (4.57, 5.68 and 7.13%) at 4, 8 and 12 months of storage followed by seed treated with neem leaf powder extract (2.12, 2.46 and 2.88%) at the same storage period respectively.

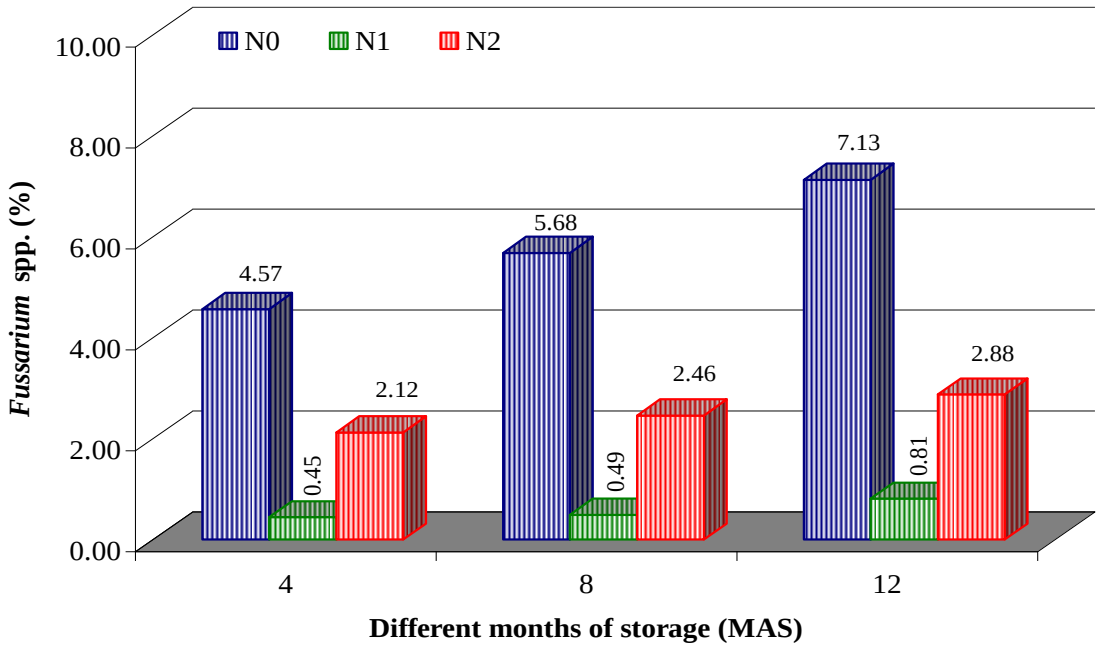
Combined effect of storage containers and seed treatments

A significant variation was found regarding prevalence of *Fusarium* spp at all the data recording period (4, 8 and 12 months of storage) due to interaction effect between storage container and seed treatments (Appendix V and Table 9). Prevalence of *Fusarium* spp (6.020, 7.720 and 9.270%) was found highest in case of T_4N_0 (gunny bag × non-treated seed) followed by T_5N_0 (earthen pot x non-treated seed) at 4, 8 and 12 months after storage. The treated seeds with provax-200 and stored in plastic container showed lowest incidence of *Fusarium* spp at 4, 8 and 12 months of storage (0.13, 0.15 and 0.17%, respectively) due to low moisture content.



Pc: Plastic container; Pb: Plastic bag; Pgb: Polythene coated gunny bag; Gb: Gunny bag and Ep: Earthen pot

Fig. 14. Effect of different storage containers on the prevalence of *Fusarrium* spp in storage okra seeds (%) at different storage periods



N_0: Control (Non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

Fig. 15. Effect of seed treatments on the prevalence of *Fusarrium* spp in storage okra seeds (%) at different months of storage

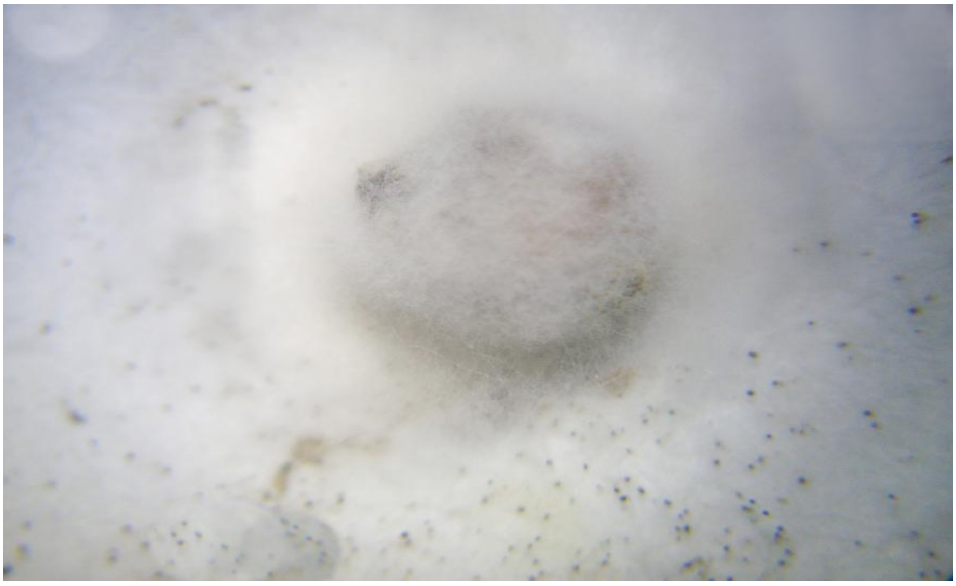


Fig. 16 : *Fusarium* spp infected okra seed under stereo – microscop (40 x)

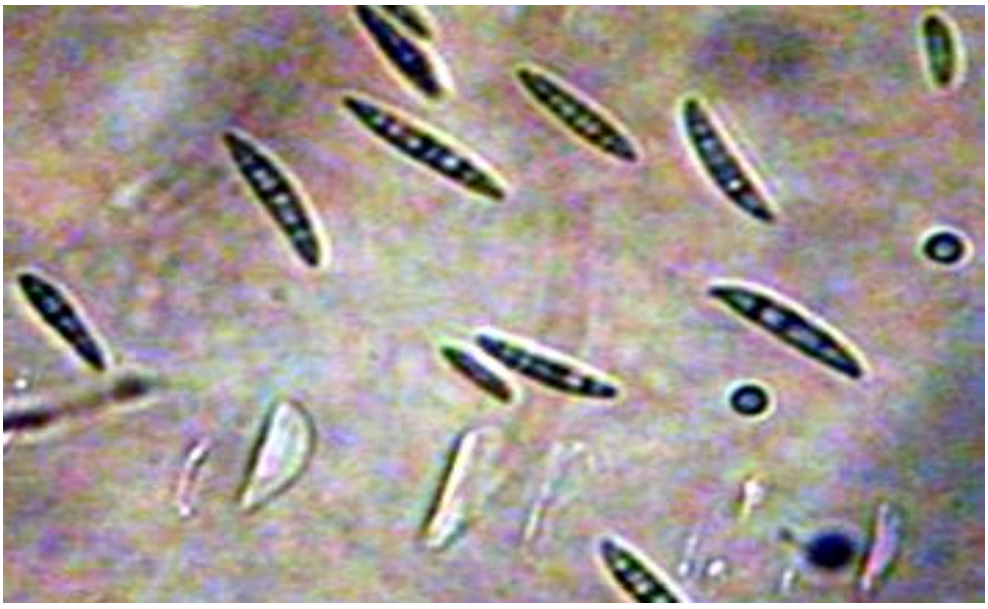


Fig. 17 : Conidiophores and conidia of *Fusarium* spp under compound microscope (40 x)

4.2.2 Prevalence of *Chaetomium globosum*

Efect of storage containers

The difference in prevalence of *Chaetomium globosum* was statistically significant among the storage containers at all the storage period (Appendix V, Fig. 17 and Fig 20-21). Prevalence of *Chaetomium globosum* (16.40, 16.44 and 17.63%,

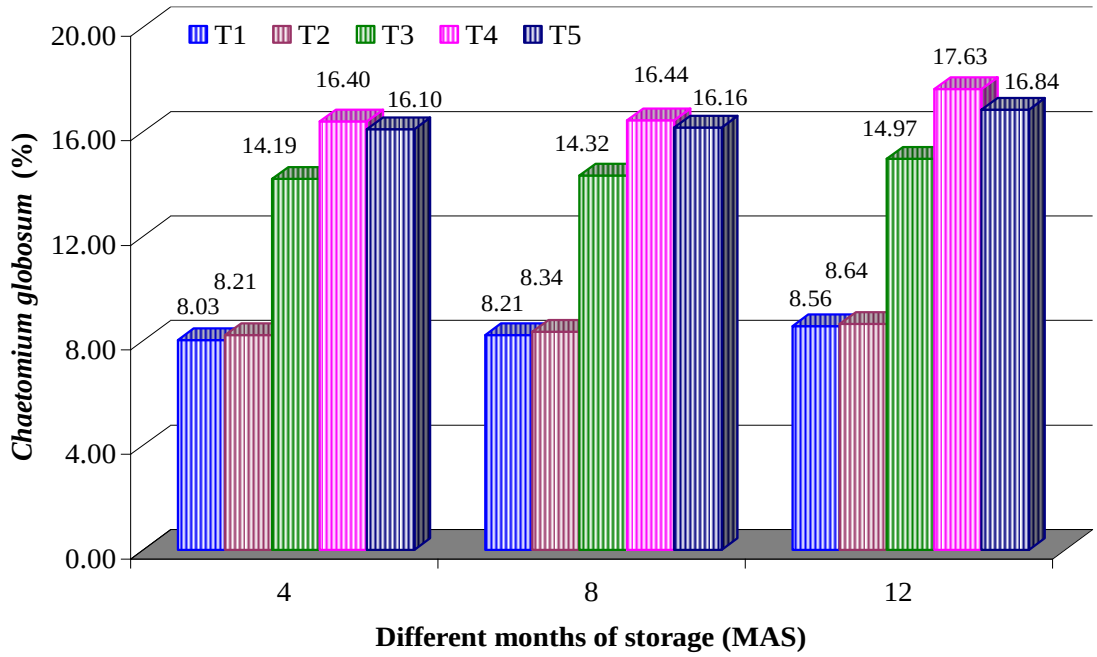
respectively) was found highest in those storage seeds which were stored in gunny bags at 4, 8 and 12 months of storage due to high moisture followed by earthen pot at those storage period (16.10, 16.16 and 16.84%, respectively).

Effect of seed treatments

Effect of seed treatments on the prevalence of *Chaetomium globosum* was significantly influenced at 4, 8 and 12 months of storage (Appendix V, Fig. 19 and Fig 20–21). In this study showed that non treated seeds was highly affected by *Chaetomium globosum* (9.61, 10.06 and 10.41% respectively) due to more moisture at 4, 8 and 12 months of storage followed by seed treated with neem leaf powder extract (3.16, 3.59 and 3.92%, respectively) at those storage period respectively.

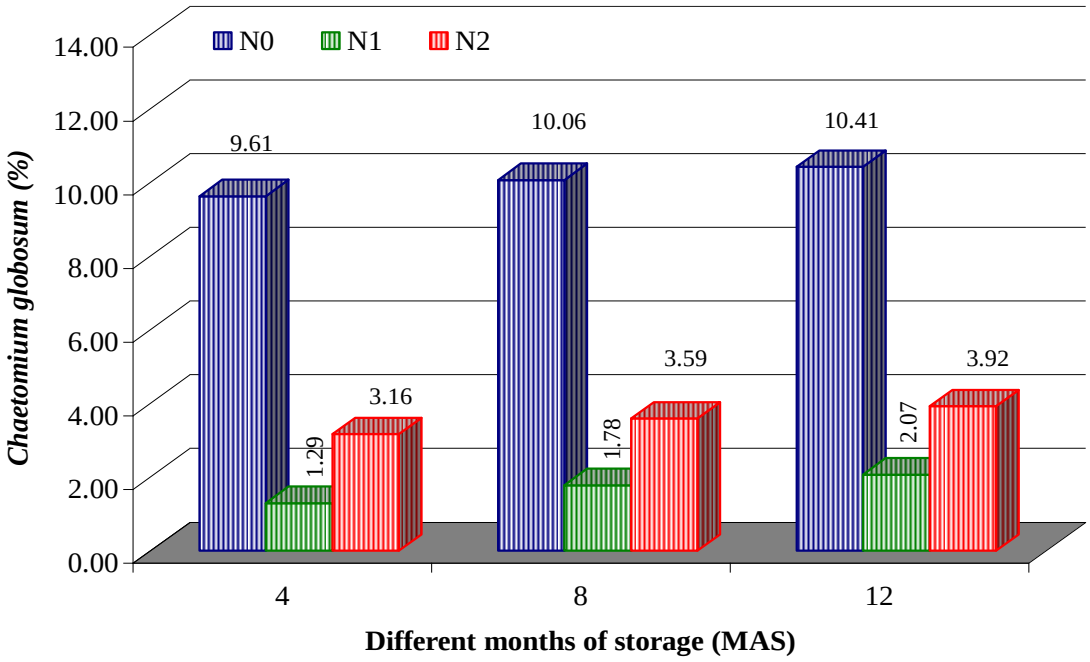
Combined effect of storage containers and seed treatments

The difference in prevalence of *Chaetomium globosum* at the storage period of 4, 8 and 12 months was statistically significant due to interaction effect between storage container and seed treatments (Appendix V, Table 9 and Figure 20–21). The prevalence of *Chaetomium globosum* where maximum incidence (11.14, 11.53 and11.96%) was obtained from the T_4N_0 (gunny bag × non treated seed) followed by the incidence (10.06, 10.13 and 10.25%) of T_5N_0 (earthen pot x non treated seed). Minimum incidence (0.720, 1.910 and 2.010%) was recorded by T_1N_1 (plastic container × seed treated with provax-200) due to low moisture content.



Pc: Plastic container; Pb: Plastic bag; Pgb: Polythene coated gunny bag; Gb: Gunny bag and Ep: Earthen pot

Fig. 18. Effect of different storage containers on the prevalence of *Chaetomium globosum* in storage okra seeds (%) at different months of storage



N_0: Control (Non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

Fig. 19. Effect of seed treatments on the prevalence of *Chaetomium globosum* in storage okra seeds (%) at different months of storage

Table 9.Interaction effect of different storage containers and seed treatments on the prevalence of *Fusarium* spp and *Chaetomium globosum* during the different period (months) of seed storage

Treatments	*Fusarium* spp at different months of storage			*Chaetomium globosum* at different months of storage		
	4	**8**	**12**	**4**	**8**	**12**
$T_1 \times N_0$	2.490 fg	2.850 f	5.140 e	7.790 e	8.410 d	8.740 d
$T_1 \times N_1$	0.130 k	0.150 l	0.170 l	0.720 l	1.910 g	2.010 ij
$T_1 \times N_2$	1.120 i	1.400 h	1.830 i	3.350 g	3.370 f	3.410 g
$T_2 \times N_0$	3.930 d	4.600 d	5.820 d	9.400 d	9.880 c	9.940 c
$T_2 \times N_1$	0.220 k	0.370 k	0.370 l	1.350 j	1.370 h	1.760 j
$T_2 \times N_2$	1.690 h	1.940 g	2.390 h	3.600 f	3.860 e	3.910 f
$T_3 \times N_0$	4.610 c	6.260 c	7.270 c	9.680 c	10.34 b	11.16 b
$T_3 \times N_1$	0.200 k	0.260 kl	0.700 k	1.370 j	1.700 g	1.800 j
$T_3 \times N_2$	2.790 e	2.790 f	2.810 g	3.350 g	3.400 f	3.950 f
$T_4 \times N_0$	6.020 a	7.720 a	9.270 a	11.14 a	11.53 a	11.96 a
$T_4 \times N_1$	0.850 j	1.020 i	1.700 i	1.050 k	1.890 g	2.160 i
$T_4 \times N_2$	2.620 f	3.330 e	3.650 f	2.820 h	3.600 ef	3.920 f
$T_5 \times N_0$	5.780 b	6.970 b	8.160 b	10.06 b	10.13 bc	10.25 c
$T_5 \times N_1$	0.830 j	0.660 j	1.110 j	1.980 i	2.010 g	2.600 h
$T_5 \times N_2$	2.390 g	2.840 f	3.710 f	2.690 h	3.720 e	4.410 e
$LSD_{(005)}$	**0.1668**	**0.1582**	**0.2042**	**0.2416**	**0.2888**	**0.3335**
CV (%)	**4.19**	**3.26**	**3.40**	**3.06**	**3.36**	**3.65**

Pc: Plastic container; Pb: Plastic bag; Pgb: Polythene coated gunny bag; Gb: Gunny bag and Ep: Earthen pot

Fig.20. *Chaetomium globosum* under compound microscope (10x)

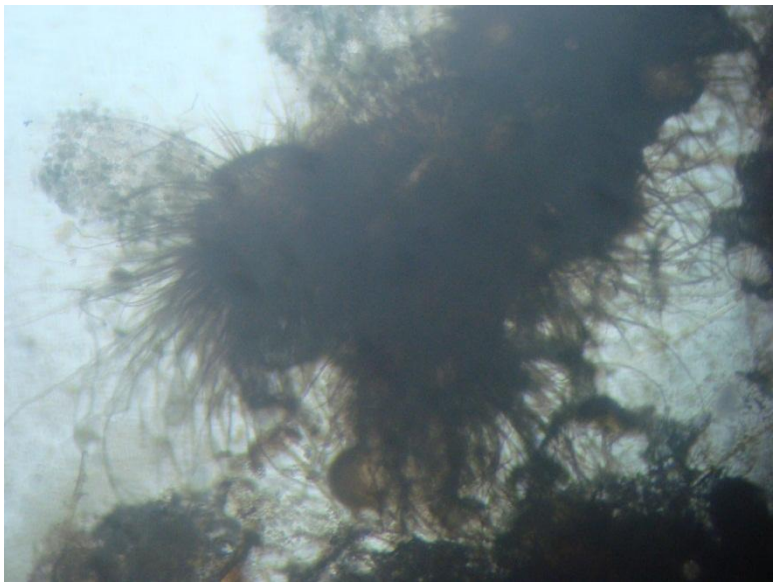


Fig. 21. *Chaetomium globosum* under compound microscope (40x)

4.2.3 Prevalence of *Aspergillus flavus*

Effect of storage containers

Prevalence of *Aspergillus flavus* in okra seed during the storage affected significantly due to various storage container treatments at different period of storage. (Appendix VI, Table 10 and Fig. 22–23). Seed stored in gunny bag (T_4)

showed highly significant prevalence (6.517, 7.027%) of *Aspergillus flavus* at 4 and 8 months of storage but in both the treatments gunny bag and earthen pot produced maximum and statistically identical incidence of *Aspergillus flavus* at 12 months of storage (8.133 and 8.28% respectively) where $T_5 > T_4$. Among the container treatments, plastic container showed significantly lower prevalence (3.13, 4.20 and 4.410%) of *Aspergillus flavus* followed by plastic bag (3.77, 4.73 and 6.24%) at 4, 8 and 12 months of storage, respectively.

Effect of seed treatments

Effect of different seed treatments on the prevalence of *Aspergillus flavus* was significantly influenced at 4, 8 and 12 months of storage (Appendix V, Table 11 and Fig 22–23). In this study showed that without treated seed had highly affected by *Aspergillus flavus* (10.30, 12.38 and 14.56 %, respectively) due to more moisture at 4, 8 and 12 months of storage followed by seed treated with neem leaf powder extract (3.672, 4.094 and 4.206% respectively) at those storage period .

Combined effect of storage containers and seed treatments

A significant variation was found regarding incidence of *Aspergillus flavus* at all the data recording period (4, 8 and 12 months of storage) due to combine effect between storage containers and seed treatments (Appendix Vl, Table 12 and Fig. 22-23). Prevalence of *Aspergillus flavus* was maximum (14.26, 15.69 and 18.26%) in those non-treated seeds which was stored in gunny bag (T_4N_0) followed by T_5N_0 (earthen pot x non-treated seed) at 4, 8 and 12 months of storage. The treated seeds with provax-200 and stored in plastic container (T_1N_1) showed minimum incidence of *Aspergillus flavus* at 4, 8 and 12 months of storage (0.520,0.540 and 0.550%, respectively) due to low moisture content.

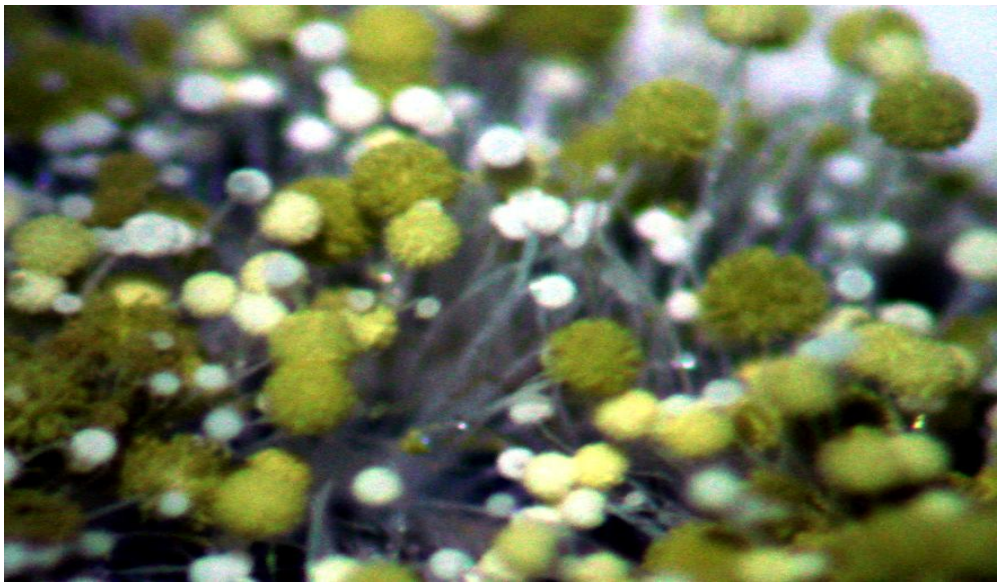


Fig .22. *Aspergillus flavus* infected okra seed under stereo – microscope (40x)

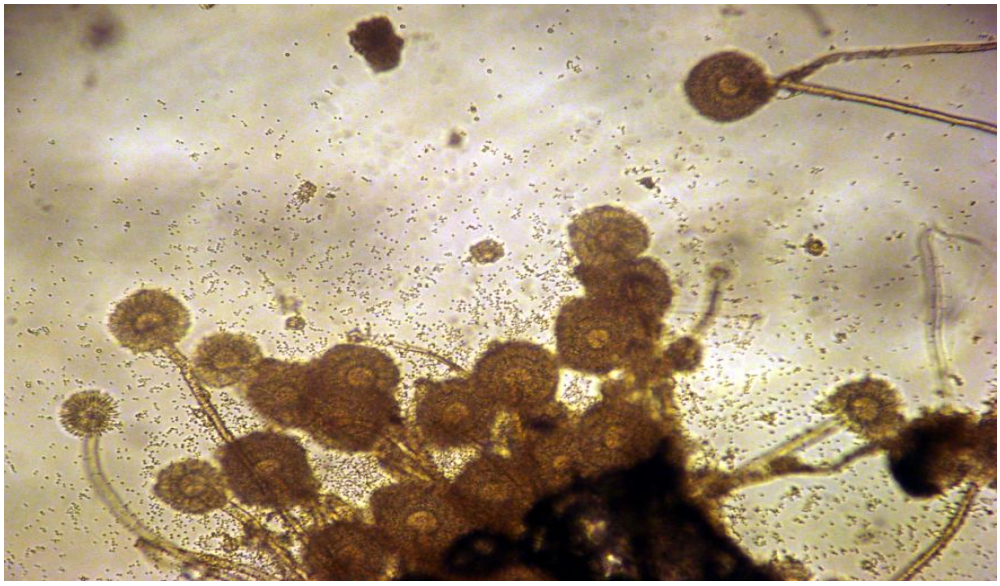


Fig .23. Conidiophores and conidia of *Aspergillus flavus* under compound microscope (40x)

4.2.4 Prevalence of *Aspergillus niger*

Effect of storage containers

Prevalence of *Aspergillus niger* was statistically significant among the storage containers at all the storage period (Appendix VI, Table 10 and Figure 24–25). Prevalence of *Aspergillus niger* (6,730, 7.024 and 7.85%, respectively) was found

highest in those storage seeds which were stored in gunny bag at 4, 8 and 12 months of storage due to high moisture followed by earthen pot at those storage period (5.647, 6.620 and 7.34, respectively).

Effect of seed treatments

Effect of seed treatments on the prevalence of *Aspergillus niger* was significantly influenced at 4, 8 and 12 months of storage (Appendix Vl, Table 11 and Figure 24–25). In this study showed that non-treated seed had highly affected by *Aspergillus niger* (9.278, 11.70 and 15.11%, respectively) due to more moisture at 4, 8 and 12 months of storage followed by seed treated with neem leaf powder extract (3.832, 4.258 and 4.52%, respectively) at those storage period. On the other hands provax-200 treated okra seeds registered the minimum incidence of *Aspergillus niger* (1.36, 1.558 and 1.89%)

Combined effect of storage containers and seed treatments

A significant variation was found regarding incidence of *Aspergillus niger* were at all the data recording period (4, 8 and 12 months of storage) due to combine effect between storage containers and seed treatments (Appendix Vl, Table 12 and Figure 24-25). Prevalence of *Aspergillus niger* was maximum (13.67, 14.22 and 16.63%) in those non treated seeds which was stored in gunny bag (T_4N_0) followed by T_5N_0 (earthen pot x non treated seed) at 4, 8 and 12 months of storage. The treated seeds with provax-200 and stored in plastic container (T_1N_1) showed minimum incidence of *Aspergillus niger* at 4, 8 and 12 months of storage (0.870, 0.900 and 1.350%, respectively) due to low moisture content.

Table 10. Effect of different storage containers on the prevalence of *Aspergillus flavus* and *Aspergillus niger* during the different period (months) of seed storage

Treatments	*Aspergilus flavus* at different months of storage			*Aspergilus niger* at different months of storage		
	4	**8**	**12**	**4**	**8**	**12**
T_1 (pc)	3.313 e	4.210 e	4.410 d	3.313 e	4.470 e	5.97 b
T_2 (pb)	3.767 d	4.733 d	6.240 c	3.863 d	4.970 d	6.32 a
T_3 (pgb)	5.050 c	6.243 c	6.580 b	4.563 c	6.117 c	7.62 a
T_4 (gb)	6.517 a	7.027 a	8.133 a	6.730 a	7.023 a	7.85 a
T_5 (ep)	5.873 b	6.757 b	8.283 a	5.647 b	6.620 b	7.34 a
$LSD_{(005)}$	**0.2065**	**0.2195**	**0.2378**	**0.1801**	**0.3766**	**0.8259**
CV (%)	**4.37**	**3.92**	**3.67**	**3.89**	**6.70**	**12.32**

Pc: Plastic container; Pb: Plastic bag; Pgb: Polythene coated gunny bag; Gb: Gunny bag and Ep: Earthen pot

Table 11. Effect of seed treatments on the prevalence of *Aspergillus flavus* and *Aspergillus niger* during the different period (months) of seed storage

Treatments	*Aspergilus flavus* at different months of storage			*Aspergilus niger* at different months of storage		
	4	**8**	**12**	**4**	**8**	**12**
N_0 (NT)	10.30 a	12.38 a	14.56 a	9.278 a	11.70 a	15.11 a
N_1 (Pvx)	0.740 c	0.904 c	1.424 c	1.36 c	1.558 c	1.89 c
N_2 (NLP)	3.672 b	4.094 b	4.206 b	3.832 b	4.258 b	4.52 b
LSD (5%)	**0.1599**	**0.1701**	**0.1842**	**0.1395**	**0.2917**	**0.6398**
CV (%)	**4.37**	**3.92**	**3.67**	**3.89**	**6.70**	**12.32**

N_0: Control (Non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

Table 12. Interaction effect of different storage containers and seed treatments on the prevalence of *Aspergilus flavus* and *Aspergilus niger* during the different period (months) of seed storage

Treatments	*Aspergilus flavus* at different months of storage			*Aspergilus niger* at different months of storage		
	4	**8**	**12**	**4**	**8**	**12**
$T_1 \times N_0$	7.000 e	8.300 e	8.840 d	5.980 e	8.820 c	12.59 c
$T_1 \times N_1$	0.520 jk	0.540 j	0.550 j	0.870 l	0.900 h	1.350 g
$T_1 \times N_2$	2.420 h	3.790 g	3.840 f	3.090 h	3.690 f	3.960 e
$T_2 \times N_0$	7.490 d	9.770 d	14.00 c	6.620 d	9.320 c	15.16 ab
$T_2 \times N_1$	0.200 k	0.680 ij	0.790 ij	1.200 k	1.750 g	2.250 f
$T_2 \times N_2$	3.610 g	3.750 g	3.930 f	3.770 g	3.840 ef	3.910 e
$T_3 \times N_0$	10.14 c	13.40 c	14.23 c	8.690 c	12.36 b	16.31 ab
$T_3 \times N_1$	0.870 ij	0.940 hi	1.090 i	1.240 jk	1.510 gh	1.600 g
$T_3 \times N_2$	4.140 f	4.390 f	4.420 e	3.760 g	4.480 de	4.910 d
$T_4 \times N_0$	14.26 a	15.69 a	18.26 a	13.67 a	14.22 a	16.63 a
$T_4 \times N_1$	1.100 i	1.100 h	1.710 h	1.950 i	1.960 g	1.980 g
$T_4 \times N_2$	4.190 f	4.290 f	4.430 e	4.570 f	4.890 d	4.930 d
$T_5 \times N_0$	12.61 b	14.76 b	17.46 b	11.43 b	13.80 a	14.84 b
$T_5 \times N_1$	1.010 i	1.260 h	2.980 g	1.540 j	1.670 g	2.290 f
$T_5 \times N_2$	4.000 f	4.250 f	4.410 e	3.970 g	4.390 de	4.880 d
$LSD_{(005)}$	**0.3576**	**0.3803**	**0.4118**	**0.312**	**0.6522**	**1.431**
CV (%)	**4.37**	**3.92**	**3.67**	**3.89**	**6.70**	**12.32**

Pc: Plastic container; Pb: Plastic bag; Pgb: Polythene coated gunny bag; Gb: Gunny bag and Ep: Earthen pot

N_0: Control (non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

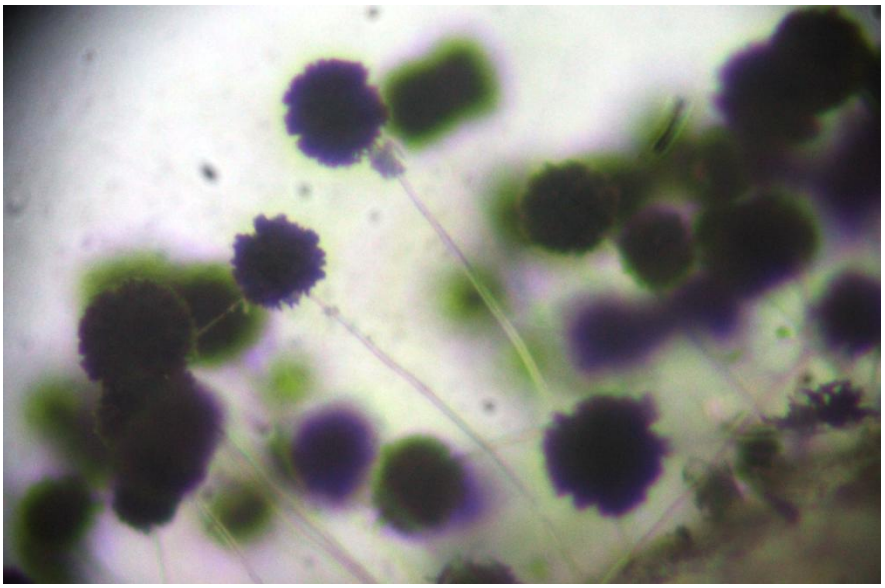


Fig. 24. *Aspergillus niger* under stereo-microscope (40x)

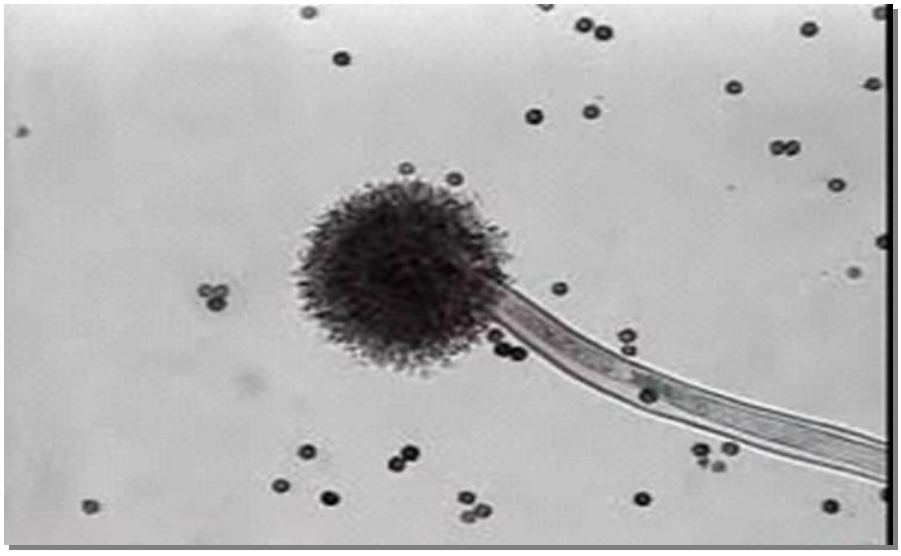


Fig. 25. Conidia and conidiophore of *Aspergillus niger* under compound microscope (40x)

4.2.5 Prevalence of *Rhizopus stolonifer*

Effect of storage containers

The difference in prevalence of *Rhizopus stolonifer* was statistically significant among the storage containers at all the storage periods (Appendix VII, Table 13 and Fig. 26). Prevalence of *Rhizopus stolonifer* (4.273 and 5.793%, respectively) was found highest in those storage seeds which were stored in gunny bag at 4 and 12 months of storage due to high moisture followed by those kept in earthen pot at

those storage period (4.160 and 5.120, respectively). But only statistically identical to the storage seeds container of gunny bag and earthen pot at 8 months of storage (4.69 and 4.70%, respectively) where earthen pot is greater than gunny bag. On the other hand, seed stored in plastic container (T_1) showed significantly the lower incidence of *Rhizopus stolonifer* (2.57, 3.59 and 4.86%) at 4, 8 and 12 months of storage, respectively .

Effect of seed treatments

Analysis of variance data on the incidence of *Rhizopus stolonifer* in okra seed at different storage period of okra seed was statistically significant (Appendix VII, Table 14 and Fig. 26). The incidence of *Rhizopus stolonifer* was maximum (7.054, 8.255 and 9.850%) in those storage seed which were not treated by any seed treatment at 4, 8 and 12 months of storage, respectively followed by treated seeds with neem leaf powder extract same storage period. At 4, 8 and 12 months of storage, provax-200 treated seed exhibited the minimum incidence of *Rhizopus stolonifer* (0.956, 1.192 and 1.678%, respectively).

Table 13. Effect of different storage containers on the prevalence of *Rhizopus stolonifer* during the different period (months) of seed storage

Treatments	*Rhizopus stolonifer* at different months of storage		
	4	8	12
T_1 (pc)	2.573 c	3.593 d	4.857 c
T_2 (pb)	3.503 b	4.080 c	4.930 bc
T_3 (pgb)	3.670 b	4.237 b	5.027 bc
T_4 (gb)	4.273 a	4.693 a	5.793 a
T_5 (ep)	4.160 a	4.696 a	5.120 b
SX	**0.06412**	**0.03944**	**0.06992**
Level of sig.	**	**	**

Pc: Plastic container; Pb: Plastic bag; Pgb: Polythene coated gunny bag; Gb: Gunny bag and Ep: Earthen pot

****= Significant at 1% level of probability**

Table 14. Effect of seed treatments on the prevalence of *Rhizopus stolonifer* during the different period (months) of seed storage

Treatments	*Rhizopus stolonifer* at different months of storage		
	4	**8**	**12**
N_0 (NT)	7.054 a	8.255 a	9.850 a
N_1 (Pvx)	0.956 c	1.192 c	1.678 c
N_2 (NLP)	2.898 b	3.332 b	3.908 b
LSD (5%)	**0.1434**	**0.08824**	**0.1564**
CV	**5.30**	**2.78**	**4.09**
Level of sig.	**	**	**

N_0: Control (Non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

****= Significant at 1% level of probability**

Combined effect of storage containers and seed treatments

A significant variation was found regarding incidence of *Rhizopus stolonifer* at all the data recording period (4, 8 and 12 months of storage) due to combined effect of storage containers and seed treatments (Appendix Vll, Table 15 and Fig 26) prevalence of *Rhizopus stolonifer* was maximum (8.260, 8.720 and 10.63%) in those non-treated seeds which was stored in gunny bag (T_4N_0) followed by T_5N_0 (earthen pot x non treated seed) at 4, 8 and 12 months of storage. The treated seeds with provax-200 and stored in plastic container (T_1N_1) showed lowest incidence of *Rhizopus stolonifer* at 4, 8 and 12 months of storage (0.590, 0.900 and 1.570%, respectively) due to low moisture content.

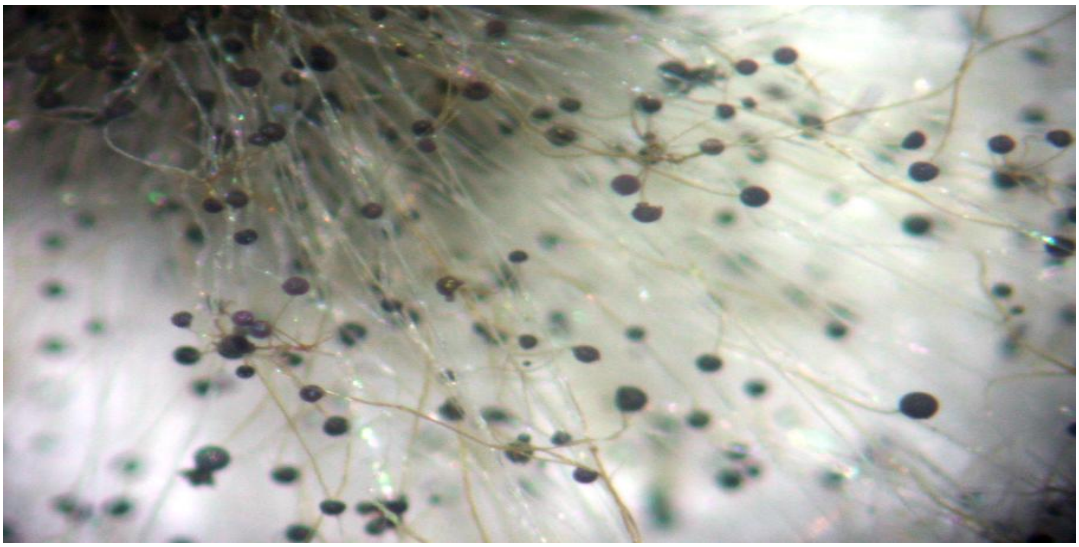


Fig. 16. Sporangia of *Rhizopus* spp isolated from okra seed under compound microscope (40x)

4.2.6 Prevalence of *Curvularia* spp

Effect of storage containers

Prevalence of *Curvularia* spp was statistically significant among the storage containers at all the storage period (Appendix VII, Fig. 27 and Fig 29-30). Prevalence of *Curvularia* spp (3.90, 4.81 and 5.19%, respectively) was found highest in those storage seeds which were stored in gunny bag at 4, 8 and 12 months of storage due to high moisture followed by earthen pot at same storage period (3.69, 4.26 and 4.62%, respectively).

Effect of seed treatments

Calculating data on the prevalence of *Curvularia* spp different storage period of okra seeds were significantly influenced by the seed treatment in this study (Appendix VII, Fig. 12 and Plate 17). Among the seed treatment, non- treated seed produced significantly the highest incidence (6.61, 7.40 and 8.20%) followed by neem leaf powder extract (2.95, 3.23 and 3.80, respectively) at 4, 8 and 12 months of storage. On the other hand at 4, 8 and 12 months of storage, provax-200 treated seed exhibited the minimum incidence of *Curvularia* spp (1.20, 1.58 and 2.04%) at 4, 8 and 12 months of storage, respectively.

Combined effect of storage containers and seed treatments

A significant variation was observed in prevalence of *Curvularia* spp were at all the data recording period (4, 8 and 12 months of storage) due to combined effect between storage containers and seed treatments (Appendix VII, Table 15 and Fig. 29-30). Prevalence of *Curvularia* spp was highest (8.210 and 9.090%) in those non treated seeds which was stored in gunny bag (T_4N_0) followed by T_5N_0 (earthen pot x non-treated seed) at 8 and 12 months of storage. But at 4 months of storage, treatment T_5N_0 (earthen pot × non treated seed) showed significantly the highest (6.820%) followed by T_4N_0 (6.710%, respectively).

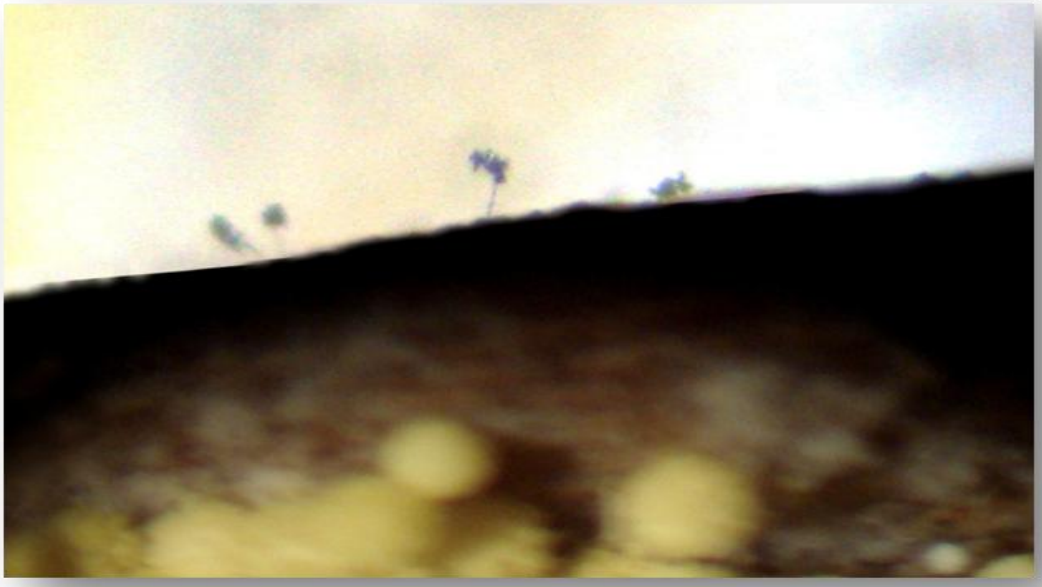
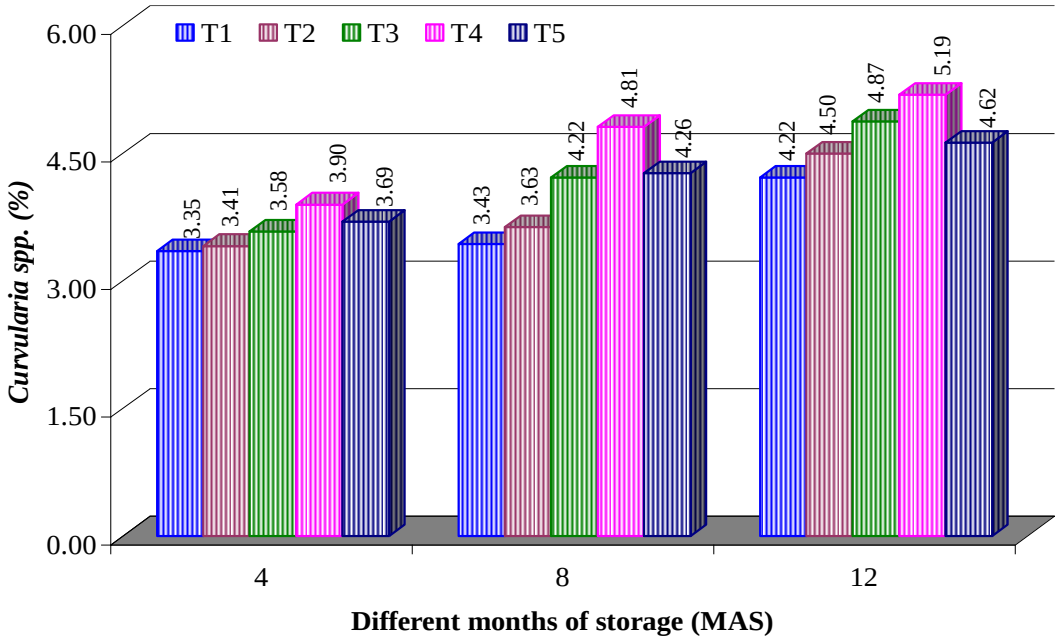
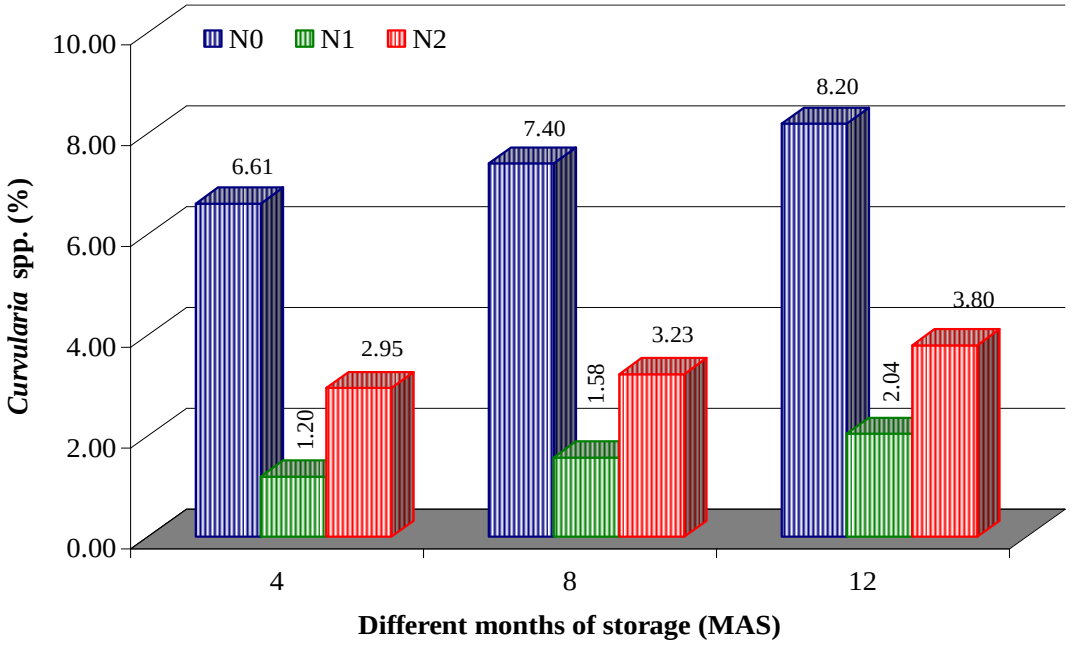


Fig.29 : *Curvularia* spp infected okra seeds under stereo –microscope (40x)



Pc: Plastic container; Pb: Plastic bag; Pgb: Polythene coated gunny bag; Gb: Gunny bag and Ep: Earthen pot

Fig. 27. Effect of different storage containers on the prevalence of *Curvularia* spp in storage okra seeds (%) at different months of storage



N_0: Control (Non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

Fig. 12. Effect of seed treatments on the prevalence of *Curvularia* spp in storage okra seeds (%) at different months of storage

Table 15. Interaction effect of different storage containers and seed treatments on the prevalence of *Rhizopus stolonifer* and *Curvularia* spp during the different period (months) of seed storage

Treatments	*Rhizopus stolonifer* at different months of storage			*Curvularia* spp at different months of storage		
	4	**8**	**12**	**4**	**8**	**12**
$T_1 \times N_0$	5.420 d	7.370 c	9.100 d	6.320 b	6.410 d	7.820 c
$T_1 \times N_1$	0.590 k	0.900 i	1.570 i	0.800 f	0.900 h	1.200 i
$T_1 \times N_2$	1.710 g	2.510 f	3.900 f	2.930 c	2.980 f	3.630 e
$T_2 \times N_0$	6.020 c	7.490 c	9.480 c	6.620 ab	7.040 c	7.800 c
$T_2 \times N_1$	0.780 jk	1.030 hi	1.250 i	0.810 f	1.000 h	1.920 h
$T_2 \times N_2$	3.710 e	3.720 d	4.060 f	2.800 c	2.850 f	3.770 de
$T_3 \times N_0$	7.330 b	8.550 b	9.970 b	6.570 ab	7.630 b	8.540 b
$T_3 \times N_1$	0.930 ij	1.170 h	1.920 h	1.190 e	1.520 g	2.020 gh
$T_3 \times N_2$	2.750 f	2.990 e	3.190 g	2.970 c	3.500 e	4.060 d
$T_4 \times N_0$	8.260 a	8.720 b	10.63 a	6.710 a	8.210 a	9.090 a
$T_4 \times N_1$	1.150 hi	1.660 g	2.240 h	1.860 d	2.710 f	2.760 f
$T_4 \times N_2$	3.410 e	3.700 d	4.510 e	3.120 c	3.510 e	3.720 de
$T_5 \times N_0$	8.240 a	9.147 a	10.07 b	6.820 a	7.730 b	7.730 c
$T_5 \times N_1$	1.330 h	1.200 h	1.410 i	1.320 e	1.750 g	2.310 g
$T_5 \times N_2$	2.910 f	3.740 d	3.880 f	2.940 c	3.310 e	3.830 de
$LSD_{(005)}$	**0.3208**	**0.1973**	**0.3498**	**0.2983**	**0.2936**	**0.3615**
CV (%)	**5.30**	**2.78**	**4.09**	**5.00**	**4.34**	**4.63**

Pc: Plastic container; Pb: Plastic bag; Pgb: Polythene coated gunny bag; Gb: Gunny bag and Ep: Earthen pot

N_0: Control (Non-treated); N_1: Seed treated with provax-200 and N_2: (NLP) Seed treated with neem leaf powder extract

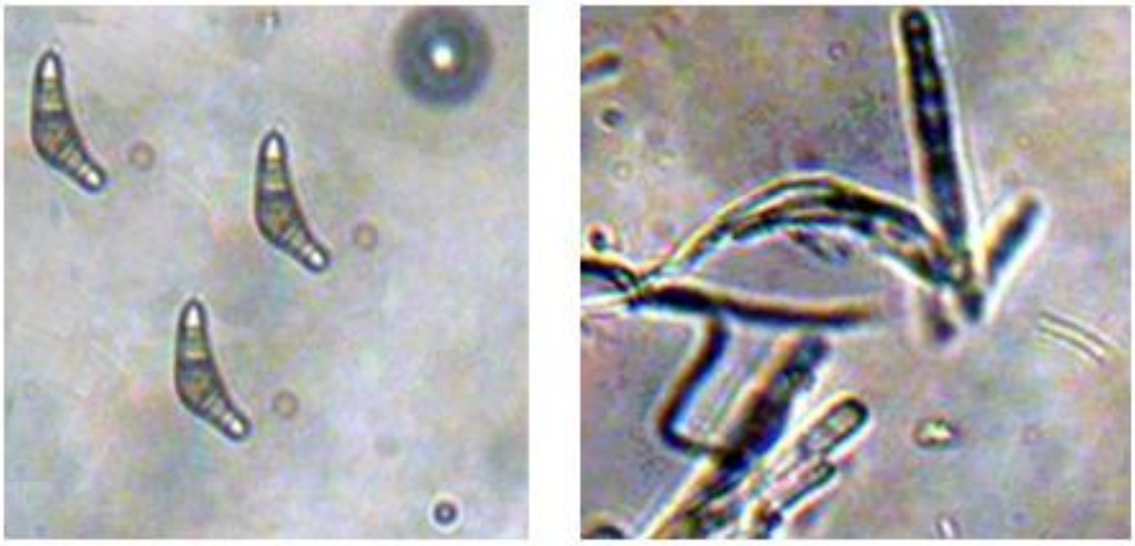


Fig.30. Conidia, conidiophores and mycelia of *Curvularia* spp under compound microscope (40x)

The treated seeds with provax-200 and stored in plastic container (T_1N_1) showed minimum incidence of *Curvularia* spp at 4, 8 and 12 months of storage (0.800, 0.900 and 1.200, respectively) due to low moisture content.

CHAPTER V

DISCUSSION

Investigations were carried out to evaluate the seed quality of okra seeds in relation to quality attributes and prevalence of seed borne fungi as influence by storage container and seed treatment.

In case of quality attributes due to storage container, all the characters were significantly affected, whereas the seeds stored in gunny bag were highly affected by moisture due to high keeping capability of moisture than other storage container (plastic container, poly bag, polythene coated gunny bag and earthen pot) which ultimately reduced the seed longevity. However, moisture content of seeds stored in polythene coated gunny bag, gunny bag and earthen pot were much higher than the recommended (10%) standard (Annon, 2006). Besides, lower moisture content help to maintain the seed quality during storage such seeds stored in plastic container accumulate the lower moisture. These findings are supported by Fabunmi (2009), Islam (2006), Anon (2006), Anam et al. (2002) and Agrawal (1992).

From the observation of the results regarding shoot length, significant reduction was found in all the storage containers with the increase in storage period and statistically similar observation was also obtained in case of seed germination study. Root length was the longest also in plastic container which might be due to the longest shoot and effectiveness of lower moisture content in the seeds which showed the increased number of germination as well as of healthy seedlings resulting the longest root which was similar with the findings of Anam et al. (2002), Ghimire (2003), Pandita and Randhawa (1992).

Present findings also revealed that provax-200 showed the higher germination rate including lower infestation of fungi species. This result corroborates with the findings of Adebisi (2012), Fagbohun and Faleye (2012).

In case of prevalence of seed borne fungi associated with the okra seeds of were namely *Fusarium* spp, *Chaetomium globosum*, *Aspergillus flavus*, *Aspergillus niger*, *Rhizopus stolonifer* and *Curvularia* spp. In case of different storage containers, the prevalence of seed borne fungi of okra were statistically significant and gunny bag seeds showed the highest prevalence by seed borne fungi and relatively lower prevalence were observed in the seeds stored in the plastic container due to lower moisture content. It was also found that the prevalence of seed borne fungi significantly increased with the increase in storage period due to increase in moisture content. From the obtained result, it was found that higher percentage of seeds were significantly infected by *Fusarium* spp (4.87%) when stored in gunny bag while seeds stored in plastic container showed relatively lower infection (2.38%) by *Fusarium* spp which were similar with the findings of other researchers (Majid, 1996; Quayum, 1999; Alam, 2001; Jamadar *et al*., 2001).

Prevalence of *Chaetomium globosum* ranges from 3.95 to 5.00%, 4.56 to 5.67% and 4.72 to 6.01% at 4, 8 and 12 months of storage, respectively where highest prevalence was found in the seeds stored in gunny bag and lowest in plastic container. The present investigation revealed some earlier workers (Fakir, 2001; Islam (2006); Jamandar *et al*., 2001).

Similarly, prevalence of *Aspergillus flavus* varied from 3.31 to 6.52%, 4.21 to 7.03% and 4.41 to 8.28% at 4, 8 and 12 months of storage, respectively where the highest percent incidence of *Aspergillus flavus* was recorded in gunny bag and the lowest was in plastic container due to the variation in moisture content and

temperature. Similarly, Majid (1996), Alam(2001), Islam (2006) and Fakir (2011) found same kind of observations with the present findings.

Statistically similar effect was also obtained in the prevalence of *Aspergillus niger* where the prevalence was minimum (3.31%, 4.47% and 5.97%) in plastic container due to lower moisture and the maximum (6.73%, 7.02% and 7.34%) was in gunny bag due to higher moisture. Islam (2006) found the incidence statistically significant where *Aspergillus niger* infection was minimum in tin container while the maximum was in gunny bag.

Similarly, in case of incidence of *Rhizopus stolonifer*, it was maximum and statistically identical in the seeds of gunny bag and earthen pot at 4 months of storage (4.27 and 4.16%, respectively) and 8 months of storage (4.69 and 4.70%, respectively). At 12 months of storage, only gunny bag stored seeds showed significantly the maximum prevalence (5.79%) of *Rhizopus stolonifer*. Seeds stored in plastic container showed significantly the minimum incidence of *Rhizopus stolonifer* (2.57, 3.59 and 4.86%) at 4, 8 and 12 months of storage, respectively. The findings of the present study was also similar to the study of Majid(1996), Quayum(1999), Alam(2001), Jamadar *et al*.(2001) and Islam, (2006).

Plastic container was highly efficient in controlling the incidence of *Curvularia* spp while gunny bag was least in respect of that which resembled the study by Islam (2006).

In case of seed treatments, the prevalence of seed borne fungi during the storage period was highly significant when seeds were treated with provax-200 and found most efficient in reducing the fungi prevalence among the treatments.

The minimum percentage of seeds were infected by *Fusarium* spp (0.45, 0.49 and 0.81%), *Chaetomium globosum* (1.29, 1.78 and 2.07%), *Aspergillus flavus* (0.74, 0.90 and 1.42%), *Aspergillus niger* (1.36, 1.56 and 1.89%), *Rhizopus stolonifer* (4.47, 4.78 and 6.65%) and *Curvularia* spp (1.20, 1.58 and 2.04%) at 4, 8 and 12 months of storage, respectively due to low moisture and higher longevity.

Provax-200 treated okra seeds were highly effective in controlling the seed borne infection of storage condition which was followed by neem leaf powder extract in this study. Non-treated seeds were highly infected by all of those seed borne fungi discussed above due to higher moisture and lower longevity. Fungal species significantly increased with the increase in storage time which supported by the findings of Nwangburuka *et al*. (2012); Islam (2012); Akther (2008), Dawar *et al*. (2008) and Abduhu (2007).

Therefore, the plastic container for seed storage in combination with provax-200 as seed treatment showed higher efficacy among the treatments of the present study in respect of quality and longevity of seeds and in controlling seed borne fungi as well.

CHAPTER VI

SUMMARY AND CONCLUTION

The present 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 during July 2013 to June 2014 to determine the seed quality and seed health status of storage okra seeds collected from the Bangladesh Agricultural Research Institute (BARI), Joydebpur. A total 400 seeds of okra were used for each sample. The experiment compared with five storage containers namely plastic container, plastic bag, polythene coated gunny bag, gunny bag and earthen pot. Three seed treatments also including *viz.* no treatment (control), seed treated with Provax-200 and Neem leaf powder extract.

The data observed on the percent infestation of seed in relation to fungi species during the seed storage at 4, 8 and 12 months of storage through blotter method. And also observed seed quality in terms of moister content (%), germination capacity (%), shoot and root length (cm), seedling dry weight (g) and seedling vigor index (SVl) and field emergence (%) determined by standard method (ISTA 2006).

The stored seed influenced by various storage container was statistically significant at all the characters studied during the study except shoot length of seedling at 8 months of storage. As a result, moisture content was its maximum in Gunny bag at 4 MAS and it increased at 12 MAS while Plastic container showed minimum MC at 4 MAS and increase at 12 MAS. On the other hand, plastic container showed significantly the maximum germination, longest shoot and root, highest SVI, highest weight of dry seedling and the maximum filed emergence at 4 MAS while they decreased that level of performances.

A significant decrease resulted also in the storage containers regarding prevalence of seed borne fungi species where provax-200 treated seeds showed significantly

the minimum percentage of seeds infested by the prevalence of *Fusarium* spp, *Chaetomium globosum*, *Aspergilus flavus*, *Aspergilus niger Rhizopus stolonifer*, *and Curvularia* spp at 4, 8 and 12 months of storage, respectively. In similar observation, seed infestation was its maximum in the seeds stored in gunny bag by the prevalence of *Fusarium* spp, *Chaetomium globosum Aspergilus flavus*, *Aspergilus niger*, *Rhizopus stolonifer* and *Curvularia* spp at 4, 8 and 12 months of storage, respectively. However, earthen pot was greater infestation than gunny bag to infeste the storage seeds of okra by *Aspergilus flavus* at 12 MAS.

In the combine effect of storage conditions, storage containers and seed treatment, seeds treated with provax-200 were found compatible with plastic containers, recorded higher seed quality and lowest pathogenic fungus infestation and negative relationship observed when compared to other storage containers and treatment combinations throughout the storage period.

From the above observation six different species under five genera of seed borne fungi namely *Fusarium* spp, *Chaetomium globosum*, *Aspergilus flavus*, *Aspergilus niger*, *Rhizopus stolonifer*, *and Curvularia* spp were detected in five different types of storage containers. Therefore the seed treated with provax-200, stored in the plastic container would be the best for longevity of okra seeds in storage.

The following recommendation may be suggested for the present study-

1. Further study may be needed to ensure the performance of quality attributes and incidence of seed borne fungi on okra seed during storage.
2. More storage containers and seed treatments may be included for further study to ensure a better storage container and seed treating agent in case of okra.

REFERENCES

Abduhu, M. 2007. Quality of okra seed collected from farmers and control of seed borne pathogens by seed treatment. MS Thesis, Department of Plant Pathology. Sher–e–Bangla Agricultural University, Dhaka. pp. iv.

Abdul–Rafiu, A. M. 2007. Effect of variety and seed treatment on Sitophilus zeamais infestation and maize seed quality. M. Agric. Dissertation. Department of Plant Breeding and Technology, University of Agriculture, Abeokuta, Nigeria. pp. 83.

Abdul-Baki A, Anderson JD. 1973. Vigor determination in Soybean seed by multiple criteria. Crop Sci. 13: 630–633.

Adebisi, M. A. 2012. Efficacy of plant and chemical materials on quality of okra seed stored under natural ageing conditions. Nigerian J. Hort. Sci., 17: 157–168.

Adebisi, M. A. and K. O. Oyekale. 2005. Effect of seed treatments and storage containers on the maintenance of viability of okra seed. ASSET J. Series: A. 5(1): 81–89.

Adebisi, M. A., I. O. Daniel and M. O. Ajala. 2003. Storage life of soybean seed after seed dressing. J. Trop. Agric. (India). 42: 3–7.

Ahmed, N. 2011. Seed borne fungi of lentil and management of stemphylium blight of lentil, M.S. thesis, Dept. of Plant Pathology, BAU, Mymensingh pp. 56.

Aktaruzzaman, M., M. O. K. Azad, A. K. R. Shiton, M. Mottakina Akter, U. Habiba. 2010. Effect of biotic and abiotic factors on quality of okra seed kept in different containers. Bangladesh Res. Pub. J., 3(2): 1012–1020.

Akter, M. 2001. Bio–control of seed and seedling diseases of okra and cucurbits. MS Thesis, Department of Plant Pathology, BAU, Mymensingh. pp. 21–29.

Akter, N. 2008. Effect of plant extract on the management of seed borne fungal diseases of Okra. M. S. thesis, Dept. of Plant Pathology, BAU, Mymensingh. pp. 36 & 74.

Aladele, S. E., Ariyo, O.J. and Lapena, R.de. 2008. Genetic relationships among West African okra (Abelmoschus caillei) and Asian genotypes (Abelmoschus esculentus) using RAPD. Indian J. Biotechnol, 7 (10):1426–1431.

Alam, A. A. K. M. and A. Q. M. Bazlur Rashid, 2005. Quality of okra seeds available in the market. J. Agric. Rural Dev., 3(1&2): 111–115.

Alam, A. K. M. A. 2001. Studies on the quality of vegetable seeds available in the market, MS thesis, Department of Horticulture, BAU, Mymensingh. pp. 90.

Al–Kassim, M. Y., and M. N. Monawar. 2000. Seed borne fungi of some vegetable seeds in Gazan Province and their chemical control. Saudi. J. Biol. Sci., 7 (2): 179–184.

Al–Kassium, M. Y. 1996. Seed borne fungi of some vegetables in Saudi Arabia and their chemical control. Arab Gulf J. Sci. Res., 14 (3): 705–715.

Ambekar, J. S., A. S. Pawar and M. V. Sakhare. 2000. Bio efficacy or neem formulations and synthetic insecticides against okra fruit borer. J. Maharashtra Agril. Univ. India. 24 (3): 315–316.

Anonymous, 1976. International Rules for Seed Testing. International Seed Testing Accusation (ISTA). *Seed Sci. Tech.,* 24 (Supplement) : 29–72.

Anam, M. K., G. A. Fakir, K. M. Khalequzzaman, M. M. Hoque and A. Rahim. 2002. Effect of seed treatment on the incidence of seed borne diseases of okra. Plant Path. J., 1: 1–3.

Anonymous. 2004. Garlic tablet in Plant diseases control. A leaflet Published by IPM Lab, Dept. of Plant Pathology, BAU, Mymensingh, Bangladesh.

Arun, A., C. Rekha and A. Chitra. 1995. Effect of Alicia and extracts of garlic and Bigonia on two fungi. Indian J. Mycol. and Plant Pathol., 25(3): 316–318.

Ashrafuzzaman, H. 1991. A Text Book of Plant Pathology. pp. 374–375.

Badaru, O. 2011. The effect of *Moringa oleifera* (Moringa species) leaf extract on germination and fungal growth of *Abelmoschus esculentus* L. (Okra). B.Sc. Dissertation, Babcock University, Nigeria.

Baki, A. A. and J.D. Anderson. 1972. Physiological and biological deterioration of seeds. In seed biology, Vol. II. Academic Press, New York.

Barnett, H. L. 1967. Illustrated genera offungi imperfecti. *Burgess Publ. Co. Minneapolis, USA*. pp. 223.

BARI (Bangladesh Agricultural Research Institute). 2010. Annual progress report of okra cultivation. Bangladesh.

BBS (Bangladesh Bureau of Statistics). 2012. Summary crop statistics and crop induces (2010–11). Bangladesh Bureau of Statistics Division, Govt. of the People's Republic of Bangladesh. pp. 39. (online available at http://www.bbs.gov.bd/userfiles/Image/ArgYearBook11/Chapter–pdf).

Begum, M., S. Lokesh, R. V. Ravishankar, M. D. Shailaja, T. V. Kumar and H. S. Shetty. 2005. Evaluation of certain storage conditions for okra [*Abelmoschus esculentus* (L.) Moench] seeds against potential fungal pathogens. J. Agri. Biol., 7 (4): 550–554.

Booth, C. 1971. The genus Fusarium. Commonwealth Mycological Institute, Kew, Surrey, England.

Chowdhury, M. S. M., I. Hossain, G. A. Fakir, F. M. Aminuzzaman and M. R. Islam 2000. Tolerance of *Trichoderma harzianum* and *Gliocladium viride* to agrochemicals and their antagonistic effect on seed borne mycoflora of pigeon pea. Bangladesh J. Seed Sci. & Tech., 4 (1&2): 83–86.

Dash, S. K. and A. Narain. 1996. Efficacy of selected fungicides on seed–borne fungi and percentage of germination of diseased seeds of crops. Dept. of Plant Pathology., Orissa Univ. Agric. and Tech.,11 (2): 207–211.

Dawar, S., S. Hayat, M. Anis and M. J. Zaki. 2008. Effect of seed coating material in the efficacy of microbial antagonists for the control of root rot fungi on okra and sunflower. Pak. J. Bot., 40 (3): 1269–1278.

Doijode, S. D. 1997. Effect of storage temperature and packaging on longevity of tomato seeds. Vegetable Sci., 24: 70–72.

Ellis, M. B. 1971. Dematiaciaous Hyphomycetes. Commonwealth Mycol. Inst. Kew, Surrey, England. pp. 608.

Esuruoso, A. O. 2010. Effect of storage duration and seed invigouration treatments on quality of kenaf (*Hibiscus cannabinus* I.) seed stored under ambient conditions. Unpublished Masters of Agric. Dissertation. Department of Plant Breeding and Seed Technology, Federal University of Agriculture, Abeokuta, Nigeria. pp. 84.

Fabunmi, O. A. 2009. Effects of packaging materials and storage temperature on quality of fresh okra (*Abelmoschus esculentus*) fruit. Agricultura Tropica Et Subtropica, 42 (4): 151–156.

Fagbohun, E. D. and O. S. Faleye. 2012. Effect of storage on chemical composition and mycoflora of okra (*Abelmoschus esculentus*). Intl. J. Biosci., 2 (7): 83–89.

Fakir, G. A. 2000. An annotated list of seed borne diseases in Bangladesh. Seed Pathology Laboratory, Department of Plant pathology, Bangladesh Agricultural University Mymensingh. pp. 20.

Fakir, G. A. 2001. An annotated list of seed borne disease in Bangladesh. Seed Pathology Laboratory. Department of Plant Pathology, Bangladesh Agricultural University. Mymensingh, pp. 41.

Fernandes, M., C. A., Do., O. C. Almeida, R. Conha and C. F. Robbs. 1992. Preliminary studies of health testing in okra seed from different municipalities of Rio de Janerio State. Rev. Plant Pathol., 72(10): 796.

Ghimire, S. P. 2003. Influence of locations, storage periods and packaging materials on storability of onion (*Allium cepa* L.) and okra (*Abelmoschos esculentus* L. Moench) seeds. MS Thesis, Department of Horticulture, IAAS, Rampur, Chitwan, Nepal. pp. 87.

Gopalan, C., Rama Sastri, B. V. and Balasubramanian, S. 2007. Nutritive Value of Indian Foods. National Institute of Nutrition (NIN), ICMR.

Guha, P., J. Biswas, B. K. De and A.K. Mandal. 2012. Post–harvest dry and wet physiological seed treatments for improved storability and field performance of okra (*Abelmoschus esculentus* L.). Indian J. Agril. Res., 46 (1): 16–22.

Gurjar, K. L., S. D. Singh and P. Rawal. 2004. Management of seed borne pathogens of okra with bio–agents. Plant Disease Res., (Ludhiana). 19 (1): 44–46.

Hamim, I., D.C. Mohanto, M. A. Sarker and M. A. Ali. 2014. Effect of seed borne pathogens on germination of some vegetable seeds. J. Phytopathology. Pest Managt., 1 (1): 34–51.

Islam, M. N. 2004. Efficacy of neem leam extract and ruradan 3G on soil borne nematodes of okra. MS Thesis, Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh.

Islam, M. Z. 2006. Effect of seed treatment with mehagoni seed and neem extract on the prevalence of seed borne pathogens of okra. MS Thesis, Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh.

Islam, S. 2012. Health and quality of okra seeds collected from monirampur upazilla of Jessore district and their treatment by plant extracts MS Thesis, Department of Plant Pathology Bangladesh Agricultural University, Mymensingh. pp. 76.

ISTA (International Seed Testing Association). 2006. International rules for seed testing. International Seed Testing Association, Bassersdorf, Switzerland.

Jamadar, M. M., Ashok, S., Shamrao, J., Sajjan, A. and Jahangidar, S. 2001. Studies on seed mycoflora and their effect on germination of colour graded okra [*Abelmoschus esculentus* (L.) Moench]. J. Crop Res. (Hisar), 22 (3): 479–484.

Jones, J.B., A.R. Chase, H. Bouzar and G.K. Arris. 1992. Identification of gram–negative plant pathogenic bacteria by the biological microplate system for carbohydrate utilization. Plant Pathogenic Bacteria, Versilles (France). Ed. INRA, Paris, pp. 193–194.

Karim, M. A. 2006. Fungi associated with okra seeds of three upazillas of Pabna and their control. MS Thesis, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Banglades.

Kassim, M. Y. 1996. Studies on seed borne fungi of some vegetables in Saudi Arabia and their chemical control. Arab Gulf J. Scientific Res., l4 (93): 705–715.

Keshavulu, K., N. Manohar Reddy, B. Rajeswari, M. Arun Kumar and R. Ankaiah. 2012. Effect of Seed Vigour on Field Performance and Seed Yield in Okra (*Abelmoschus esculentus* L.). Intl. J. Bio–resource & Stress Managt., 3 (1): 26–30.

Majid, M. A. 1996. Seed health status of okra of two sadar Thana of Mymensingh and Thakurgaon, MS thesis, Department of Plant Pathology, BAU, Mymensingh. pp. 104.

Mashooda, B. and S. Lokesh. 2011. Role of leaf extracts of some medicinal plants in the management of fungal diseases of okra (*Abelmoschus esculentus* (L.) Moench). Archives of Phytopathol. & Plant Protect., 44 (18): 1822–1828.

Mathur, S. B. and O. Kongsdal, 2003. Common Laboratory Seed Health Testing Methods for Detecting fungi.Pub. by ISTA, Bassersdorf, Switzerland. 357-64.

Melchior, S. J.; Custódio, C. C.; Marques, T. A.; Machado Neto, N. B. Hervesting and storage of Gabiroba (Campomanesia adamantium Canb. –Myrtaceae) and there impliction in the germination Brasileira de Sementes, Londrina, Vol. 28, no.3, pp.141–150.

Mithal, M.J. 2006. Low cost and pollution free technology against root rot of okra. (online available at www.pakistan.com).

Mohanto, D.C. 2013. Effect of seed borne fungi on germination of some vegetable seeds. MS Thesis, Department of Plant Pathology. Bangladesh Agricultural University, Mymensingh.

Moretto, K. C. K., M. Barreto and R.Y. Inoue. 1997. Survey of fungi associated with some vegetables seeds. Hort. Bras., 15 (1): 7–10.

Mossler, M. A. and E. Dunn. 2009. Florida crop pest management profile: okra. University of Florida IFAS Extension Publication. pp. 1–57.

National Research Council. 2006. "Okra". Lost Crops of Africa: Volume II: Vegetables. Lost Crops of Africa.

Nwangburuka, C. C. 2010. Morphological characterization and genetic studies in okra [*Abelmoschus esculentus* (L.0 Moench]. PhD Thesis, University of Agriculture Abeokuta, Nigeria.

Nwangburuka, C. C., K. Oyekale, C. N. Ezekiel, P. C. Anokwuru and O. Badaru. 2012. Effects of *Moringa oleifera* leaf extract and sodium hypochlorite seed pre–treatment on seed germination, seedling growth rate and fungal abundance in two accessions of *Abelmoschus esculentus* (L) Moench. Archives App. Sci. Res., 4 (2): 875–881.

Odofin, O. 2010. The effect of neem extract and sodium hypochlorite pre–treatment on germination, fungal growth and mitotic index of *Abelmoschus esculentus* L. (Okra). B.Sc. Dissertation, Babcock University, Nigeria.

Oliveira. L. M., R. L. A. Bruno, K. R. G. Silva, E. U. Alves, G. Z. Silva and A. P. Andrade. 2011. Qualidade fisiológica de sementes de Caesalpinia pyramidalis Tul. durante o armazenamento. Revista Brasileira de Sementes, Londrina, 33 (2): 289–298.

Onunkun, O. (2012) Evaluation of aqueous extracts of five plants in the control of flea beetles on okra [*Abelmoschus esculentus* (L.) Moench J. Biopest., 5 (supplementary): 62 – 67

Patel, M. M., V. P. Gohel and D. G. Vala. 2004. Control of okra wilt with chemical seed treatment. Plant Disease Res. Ludhiiana. 19 (1): 47–48.

Prasad, B. K., R. Sudhir, K. Manoj., K. Randheer, K. S. Kumar, K. R. Singh, S. Ranjan, M. Kumar and R. Kumar. 2000. Storage fungi of lady's finger seed and their significance. J. Phytol. Res., 13 (1): 65–68.

Pun, K. B., D. Sabitha, V. Valluvaparidasan and S. Doraiswamy. 1998. Studies on seed–borne nture of *Macrophomina phaseolina* in okra. Plant Dis. Res., 13 (2): 162–164.

Quayum, M. A. 1999. Influence of date of harvest on the incidence of seed borne fungal pathogens in okra, MS thesis, Plant Pathology Dept., BAU, Mymensingh. pp. l–55

Rahman, A. J. M., M. K. Islam and M. A. T. Mia. 2000. Evaluation of cleaning methods to improve the quality of farmers saved rice seed. Bangladesh J. Plant Pathology. 16 (1&2): 39–42.

Rashid, M. M. 2000. A guide book of Plant Pathology. Dinajpur, Bangladesh. pp. 86–89.

Richardson, M. J. 1990. An annotated list of seed borne diseases. 4th edition, The International Seed Testing Association, Switzerland. pp. 338.

Roy, B., B. C. Sarker, M.R. Ali, S. R. Das and M. A. S. Sayed. 2012. Seed germination and seedling growth of two vegetables in responses to aqueous extract of four herbal plant leaves. J. Environ. Sci. & Natural Resour., 5 (1): 141–150.

Salvador, B. Torres, Maryjane, D. A. Gomes, Francisca Gleiciane da Silva; Clarisse, P. Benedito, Francisco, E. C. B. Pereira. 2013. Controlled deterioration to evaluate okra seed vigor. Hortic. Bras., 31 (2): 317–321.

Sarkar, D. 2010. Study on health status of okra seeds collected from different locations of Bangladesh. Department of Plant Pathology, Sher–e–Bangla Agricultural University, Dhaka–1207.

Sastry, K .S. 2013. Seed transmitted plant virus diseases. Springer, New York, pp. 327.

Saxena, O. P. 1994. Physiological and biochemical changes in relation to storage in some crop seeds. Seed Tech. News, 29: 24.

Scalon, S. P. Q., A. A. Lima, H. Scalon Filho and M. C. Vieira. 2009. Germinação de sementes e cres cimento inicial de mudas de Campomanesia ada–mantium Camb. : Efeito da lavagem, temperatura e de bioestimulantes. Revista Brasileira de Sementes, Londrina, 31 (2): 96–103.

Shahid, A., K. Anis, S. S. Chawhan, S. Ahamad and A. Khan. 2001. Studies on seed–borne nature of Macrophomina phaseolina in okra. Annual Plant Prot. Sci., Kanpur, India. 9 (1): 152–154.

Sharma, D. K., V. K. Jain, R. Jain and N. Sharma. 2013a. Postharvest study of okra [*Abelmoschus esculentus* (L.) moench] fruits and phytopathological effect of associated micro flora. Intl. J. Innovative Res. and Rev., 1 (1): 27–34.

Sharma, D.K., V. R. Jain, R. Jain and N. Sharma. 2013. Effects of microflora associated with okra (*Abelmoschus esculentus* L.) Moench seeds and their phytopathological effects. An online Intl.. 2 (2): 39–44. Available at http://www.cibtech.org/cjm.htm

Silva, A., S. C. J. G. A. Perez and R.C. Paula. 2011. Qualidade fisiológica de sementes de Psidium cattleianum Sabine acondicionadas e armazenadas em diferentes condições. Revista Brasileira de Sementes, Londrina, 33 (2): 197–206.

Singh, A. K. and M. Kumar. 2003. Efficacy and economics of neem based products against cotton jassid, Amrasca biguttula biguttula Ishida in okra. Crop Res. Hisar., 26: 271–274.

Sultana, L. 2009. Assessment of health status of TLS of Bean, Tomato, Okra and Cowpea seeds in the markets of Bangladesh, MS Thesis, Department of Plant Pathology, BAU, Mymensingh. pp. 39

Tariq, M., S. Dawar, F. S. Mehdi and M.J. Zaki. 2006. Use of *Avicennia marina* in the control of root infecting fungi on okra and mash bean. Pak. J. Bot., 38 (3): 811–815.

Thippeswamy, B., H. V. Sowmya and M. Krishnappa. 2011. Seed borne fungi of vegetable crops in Karnataka. J. Plant Disease Sci., 6 (1): 5–10.

Valeriana, P. J. 2002. Training resource text on crop development, major economic practices, disease and insect ecology, insect pests, natural enemies and disease of okra.

Vannacci, G., C. Cristani, M. Flora, S. Marino, G. Chilosi, C. Boccongelli and P. Magroj. 2001. Effectiveness of carboxin + thiram against seed borne *Fusarium* spp in bread and durum wheat proc. International symposium, Wishaw, North Warwickshire, UK., February 2001. pp. 26–27.

Zaidan, L.B.P. and R.C. Carreira. 2008. Seed germination in Cerrado species. Brazilian Journal of Plant Physiology, Campo dos Goytacazes, 20 (2): 167–181.

APPENDICES

Appendix I. Analysis of variance for moisture content and germination percentage of okra from the seed of different months after storage (MAS) as influence by storage containers and seed treatment

Source of variation	Degrees of freedom	Moisture content (%) at different MAS			Germination (%) at different MAS		
		4	8	12	4	8	12
Factor A	4	156.239**	152.454**	176.294**	1499.094**	1761.118**	4118.303**
Factor B	2	0.034ns	0.017ns	0.046ns	55.093**	68.031**	147.663**
A×B	8	0.005*	0.006*	0.002*	0.612*	2.932*	7.327**
Error	30	0.016	0.017	0.02	1.604	1.471	0.94

Appendix II. Analysis of variance for length of shoot and root of okra seedlings from the seed of different months after storage (MAS) as influence by storage containers and seed treatment

Source of variation	Degrees of freedom	Shoot length of seedlings at different MAS			Root length of seedlings at different MAS		
		4	8	12	4	8	12
Factor A	4	75.809**	81.025**	28.786**	23.52**	27.606**	9.975**
Factor B	2	0.562**	0.315ns	0.618**	0.724**	0.625**	0.71ns
A×B	8	0.027*	0.056*	0.025*	0.046*	0.073**	0.042*
Error	30	0.065	0.026	0.026	0.038	0.023	0.596

Appendix III. Analysis of variance for seedlings vigor index and dry weight of okra seedlings from the seed of different months after storage (MAS) as influence by storage containers and seed treatment

Source of variation	Degrees of freedom	Seedlings vigor index at different MAS			Dry weight of seedlings at different MAS		
		4	8	12	4	8	12
Factor A	4	5529535.8**	3254467.5**	4202938.98**	293.703**	578.327**	221.352**
Factor B	2	146215.4**	83369.6**	122673.099**	0.027ns	0.835ns	0.02ns
A×B	8	25302.15**	3697.35*	4381.021**	0.001ns	0.53ns	0.001ns
Error	30	4855.867	1385.733	873.2	4.739	12.327	8.228

Factor A: Storage containers, **Factor B:** Seed treatment and **A×B:** Storage containers × Seed treatments

****:** Significant at 1% level, ***:** Significant at 5% level of probability and ns= non-significant

Appendix IV. Analysis of variance for field emergence of okra seedling from the seed of different months of storage (MAS) as influence by storage containers and seed treatment

Source of variation	Degrees of freedom	Field emergence at different MAS		
		4	8	12
Factor A	4	1405.472**	1756.708**	4463.581**
Factor B	2	51.124*	87.434**	97.507**
A×B	8	2.469*	6.825*	3.885*
Error	30	16.732	14.925	4.861

Appendix V. Analysis of variance for the prevalence of *Fusarium* spp and *Chaetomium globosum* in okra seed at different months after storage (MAS) as influence by storage containers and seed treatment

Source of variation	Degrees of freedom	*Fusarium* spp at MAS			*Chaetomium globosum* at MAS		
		4	8	12			
Factor A	4	5.611**	9.133**	9.417**	1.598**	1.461**	2.333**
Factor B	2	64.391**	102.892**	155.852**	285.85**	284.293**	287.949**
A×B	8	1.238**	2.271**	1.09**	1.967**	1.306**	1.445**
Error	30	0.010	0.009	0.015	0.021	0.030	0.040

Appendix VI. Analysis of variance for the prevalence of *Aspergilus flavuas* and *Aspergilus niger* in okra seed at different months of storage (MAS) as influence by storage containers and seed treatment

Source of variation	Degrees of freedom	*Aspergilus flavus* at MAS			*Aspergilus niger* at MAS		
		4	8	12	4	8	12
Factor A	4	16.617**	14.135**	22.561**	17.061**	10.618**	3.145**
Factor B	2	359.802**	526.727**	718.513**	246.161**	414.186**	760.481**
A×B	8	7.65**	8.619**	10.983**	7.967**	4.728**	3.029**
Error	30	0.046	0.052	0.061	0.035	0.153	0.736

Factor A: Storage containers, **Factor B:** Seed treatment and **A×B:** Storage containers × Seed treatments

****:** Significant at 1% level, ***:** Significant at 5% level of probability and ns= non-significant

Appendix VII. Analysis of variance for the prevalence of *Rhizopus stolonifer* and *Curvularia spp.* in okra seed at different months after storage (MAS) as influence by storage containers and seed treatment

Source of variation	Degrees of freedom	*Rhizopus stolonifer* at different MAS			*Curvularia* spp. at different MAS		
		4	8	12	4	8	12
Factor A	4	4.115**	1.923**	1.270**	0.438**	2.722**	1.235**
Factor B	2	145.573**	196.774**	267.655**	114.349**	135.309**	150.691**
A×B	8	1.458**	0.554**	0.458**	0.139**	0.289**	0.451**
Error	30	0.037	0.014	0.044	0.032	0.031	0.047

Factor A: Storage containers, **Factor B:** Seed treatment and **A×B:** Storage containers × Seed treatments

****:** Significant at 1% level, ***:** Significant at 5% level of probability and ns= non-significant

Appendix VIII. Atmospheric conditions of the storage room

Day	Room Temperature (^{0}C)		Average	Relative Humidity (%)		Average
	Maximum	Minimum		Maximum	Minimum	
18-06-13	29.5	25.4	27.5	98	83	82
19-06-13	31.4	26.1	28.7	94	73	87
20-06-13	32.7	25.8	29.3	95	70	86
21-06-13	33.0	27.2	30.1	95	70	84
22-06-13	33.5	27.0	30.3	95	72	88
23-06-13	32.0	27.4	29.7	95	84	91
24-06-13	29.0	26.2	27.6	97	88	94
25-06-13	33.2	25.7	29.5	92	67	81
26-06-13	31.0	27.0	29.0	95	79	88
27-06-13	29.5	26.2	27.9	95	83	91
28-06-13	30.1	26.0	28.0	98	87	95
29-06-13	29.2	26.0	27.6	98	86	94
30-06-13	30.0	24.0	27.0	98	93	96
01-07-13	28.0	24.0	26.0	97	90	94
02-07-13	30.0	24.6	27.3	97	78	90
03-07-13	27.5	25.0	26.3	97	92	94
04-07-13	29.8	24.7	27.3	95	79	89
05-07-13	32.2	26.0	29.1	95	65	83