SEED BORNE FUNGI ASSOCIATED WITH FOUR SELECTED INBREED RICE VARIETIES OF BANGLADESH AND THEIR INFLUENCE ON SEED HEALTH AND SEEDLING VIGOR

BY

JANNATUL KUSTARY

DEPARTMENT OF PLANT PATHOLOGY
SHER-E-BANGLA AGRICULTURAL UNIVERSITY
DHAKA-1207

JUNE, 2014

SEED BORNE FUNGI ASSOCIATED WITH FOUR SELECTED INBREED RICE VARIETIES OF BANGLADESH AND THEIR INFLUENCE ON SEED HEALTH AND SEEDLING VIGOR

BY

JANNATUL KUSTARY

REGISTRATION NO.13-05755

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

MASTER OF SCIENCE
IN
PLANT PATHOLOGY

SEMESTER: JANUARY-JUNE, 2014

Approved by:

.....
(Nazneen Sultana)
Professor
Department of Plant Pathology
Sher-e-Bangla Agricultural University
Dhaka-1207
Supervisor

.....
(Dr.Md.BelalHossain)
Associate Professor
Department of Plant Pathology
Sher-e-Bangla AgriculturalUniversity
Dhaka-1207
Co-Supervisor

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

Department of Plant Pathology
Sher-e-Bangla Agricultural University
Sher-e-Bangla Nagar, Dhaka-1207

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

CERTIFICATE

This is to certify that the thesis entitled**,** *"SEED BORNE FUNGI ASSOCIATED WITH FOUR SELECTED INBREED RICE VARIETIES OF BANGLADESH AND THEIR INFLUENCE ON SEED HEALTH AND SEEDLING VIGOR"* submitted to the Department of Plant Pathology, 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 results of a piece of *bona fide* research work carried out by **JANNATUL KUSTARY** bearing Registration No**. 13-05755** 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 been duly acknowledged.

Dated: 30 May, 2015
Place: Dhaka, Bangladesh

.....
(Nazneen Sultana)
Professor
Department of Plant Pathology
Supervisor

DEDICATED

TO

MY BELOVED PARENTS

ACKNOWLEDGEMENTS

*All the praises and gratitude are due to the **Almighty Allah**, who has kindly enabled the author to complete this thesis successfully for increasing knowledge and wisdom.*

*The author desires to express her deepest sense of gratitude, respect, profound appreciation and indebtedness to her research Supervisor, **Nazneen Sultana**, Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for her kind and scholastic guidance, valuable suggestions, inspiration, co-operation and constructive criticisms throughout the entire period of research work and the preparation of the manuscript of this thesis.*

*The author expresses heartfelt gratitude and indebtedness to her Co-supervisor, **Dr. Md. Belal Hossain**, Associate Professor, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for his co-operation, criticisms on the manuscript and helpful suggestions for the successful completion of the research work.*

Special thanks and indebtedness to all the respective teachers of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their valuable teaching, sympathetic co-operation and inspiration throughout the period of the study.

The author thankfully remembers the students of the Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their cooperation in the entire period of study. The author also extends her thanks to all the staff of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka for their help and cooperation during the research work.

The author also likes to give thanks to all of her friends for their support and inspiration throughout her study period in Sher-e-Bangla Agricultural University, Dhaka.

The author also expresses her thanks especially to Nure Alam for his cordial support, cooperation and inspiration in preparing this thesis.

Finally, the author found no words to thank her parents and her friends for their unquantifiable love and continuous support, their sacrifice, never ending affection, immense strength and untiring efforts for bringing her dream to proper shape. They were constant source of inspiration, zeal and enthusiasm in the critical moment of her studies.

The Author

SEED BORNE FUNGI ASSOCIATED WITH FOUR SELECTED INBREED RICE VARIETIES OF BANGLADESH AND THEIR INFLUENCE ON SEED HEALTH AND SEEDLING VIGOR

ABSTRACT

An experiment was conducted in the Seed Health Laboratory, Sher-e-Bangla Agricultural University; Dhaka during the period of July 2013 to June 2014 to find out the effect of seed borne fungi of four selected inbreed rice varieties namely BRRI Dhan28, BRRI Dhan55, BINA Dhan8 and NERICA 10. The experiment was carried out in Completely Randomized Design (CRD) with four replications and seeds were tested following blotter method, water ager test tube method and rolled paper towel method. Six fungal species viz. *Bipolaris oryzae, Aspergillus flavus, Aspergillus niger, Alternaria alternata. Fusarium moniliforme and Curvularia lunata* were detected from rice seed. The incidence of different pathogens varies individually and independently among the varieties of rice seed. Seed germination varied from 91 to 96% in blotter method where the highest germination was recorded from BRRI Dhan28. The range of incidence of *Alternaria alternata (*1.03-4.48%), *Aspergillus flavus* (2.24-7.42%), *Aspergillus niger* (4.22-8.20%), *Bipolaris oryzae* (0.31-8.36%), *Fusarium moniliforme* (6.42-9.38%), *Curvularia lunata* (0.24-4.49%) were found in blotter method. Among the varieties tested, the highest number of abnormal seedlings (9.34%), diseased seedlings (20.52%) and dead seeds (10.00%) were recorded from the rice variety NERICA 10. In rolled paper towel method, seed germination ranged from 89-93%, abnormal seedlings varied from 18.09 to 24.39% and dead seeds ranged from 6.75-10.25%. BRRI Dhan28 gave the highest root length (10.73 cm) and maximum vigor index (1556). Among the tested varieties BRRI Dhan28 was the most superior in terms of health and quality, whereas NERICA 10 showed lowest germination percentage with high fungal incidence. In most cases seed borne pathogens reduced the germination and produced maximum number of diseased seedlings.

LIST OF CONTENTS

LIST OF CONTENTS (Cont'd)

LIST OF TABLES

LIST OF FIGURES

LIST OF FIGURES (Cont'd)

LIST OF APPENDICES

LIST OF SYMBOLS AND ABBREVIATIONS

ABBREVIATIONS	FULL WORD
%	Per cent
et al.	And others
cv.	Cultivar (s)
spp.	Species
viz.	Namely
etc.	Etcetera
^{0}C	Degree Celsius
Cm	Centimeter
G	Gram
No.	Number
&	And
pv.	Pathovar
Lbs	Pound
i.e.	That is
Ha	Hectre
J.	Journal
df .	Degrees of freedom
Hr	Hour (s)
Cv	Cultivar (s)
T	Treatment
LSD	Least Significant Difference
CRD	Complete Randomized design

LIST OF SYMBOLS AND ABBREVIATIONS (Cont'd)

ABBREVIATIONS	FULL WORD
CV	Co-efficient of Variance
ANOVA	Analysis of variances
PDA	Potato Dextrose Agar (media)
SAU	Sher-e-Bangla Agricultural University
BAU	Bangladesh Agricultural University
IRRI	International Rice Research Institute
BBS	Bangladesh Bureau of Statistics

CHAPTER 1

INTRODUCTION

Rice (*Oryza sativa*) is one of the world's primary food crops mostly grown in tropical and sub tropical climate. It is a self pollinated cereal crops under the family of Gramineae and subfamily Oryzoidea. It is used as staple food by 60% of the world population and grows in more than 100 countries. It is also the most important cereal crops in Asia and producing about 96% of the total rice production of the world (IRRI, 2006). About 84.5% of cropped area of Bangladesh is used for rice production (BBS, 2012). It is the staple food of Bangladeshi people and it constituted about 90% of the total food grain production. It covers about 75% of the total cultivable land in Bangladesh (Ahmed *et al*. 2013). The average world yield of rice is 3.84 tons/ha (Ahmed *et al*., 2013). So the average per hectare production of rice in Bangladesh is extremely low as compared to other rice growing countries of the world. Total seasonal production of rice in Bangladesh were 2326000 metric ton in Aus season, 1, 30, 23,312 metric ton in Aman season and 1, 90, 07,207metric ton in Boro season respectively (BBS, 2013-2014).

High quality seed is not only important for increased crop production but also for proper establishment of sound seed industry in the country. Among the characteristics, purity, germination, high yielding potentiality and seed health are the most important for quality seed. Of these major characteristics of quality, seed health is immensely important. The truth "Health is Wealth" is also equally applicable to the health of seed. The most important and destructive diseases of our important crops are caused by the seed-borne pathogens. Seed health refers to whether a seed or a seed lot is infected by pathogens or not. Infected seeds fail to germinate and the pathogens from infected seeds may be transmitted to seedlings and growing plants in field and causes diseases. Therefore, it is important to know whether a seed lot is free from seed-borne infections of pathogen(s) or the lot contains pathogen(s) with its maximum acceptable limit. Pathogen free seed is the vital input in agriculture. The average yield of rice in this country is low compared to other

countries due to seed borne diseases. In Bangladesh, approximately 2.5 million tons of rice worth more than BDT 12 thousand million is lost due to seed borne pathogens (Fakir *et al*., 2003). Without improving the quality of seed, the improved technology can hardly improve the production potentially. Normally farmers do not test the quality and health status of rice seed, but so many devastating diseases can be carried by seed and there is a great possibility to remain pathogen within the seed. There are many causes of low yield of rice in Bangladesh of which disease and pest plays a major role (Fakir, 1982). Among them seed borne diseases are more destructive. Rice seeds are known to harbor a wide range of both fungi and bacteria (Neer gaard, 1977). Seed borne diseases create a great threat to the production of crops in Bangladesh.

Rice suffers from more than 60 different diseases. In Bangladesh, 43 diseases are known to occur on the rice crop. Among these diseases, 27 are seed borne of which 14 are of major importance. The infected seeds may fail to germinate and transmit disease from seed to seedling and from seedling to growing plants (Fakir *et al.*, 2002). Infected seeds germinate poorly and could be a major source of inoculums for new crops raised from them. For example, most pathogens causing abnormal seedling of rice are seed borne (Guerrero *et al.*, 1972). Seed borne pathogens affect seed quality (Khare, 1999). Islam *et al*.(2000) observed that highest lethal seed infection caused by *Fusarium moniliforme, Trichoconis padwickii* and *Curvularia* spp. About 20 species of fungal pathogens were detected from rice seed at any one time (Mew and Gonzales, 2002). The crop is affected by as many as 36 seed-borne diseases of which 31 were caused by fungi (Ou, 1985). Totally 8 genera of fungi viz., *Alternaria,Aspergillus, Bipolaris, Chaetomium, Curvularia, Fusarium, Sarocladium* and *Trichoderma* comprising twelve species were found to be associated with the seed samples. Among them, the most predominant one was *Bipolaris oryzae* (Gopalakrishnan and Valluvaparidasan, 2010). Rice seed play an important role to carry pathogen in quarantine aspect. Farmers generally use different rice varieties and face the difficulties of many diseases. In the last few years the cultivation of rice in Bangladesh increased rapidly. Seed as a

biological entity and a basic agricultural input, is the end product of plant growth by which plant life is regenerated. Plant disease causing pathogens are mostly seed borne and seed transmitted because diseases of seed, seedling, and adult plants at various growth stages there is a chance to transmit new race of the pathogen in the country by rice seed. So, assessment of the seed health standard of rice is very important for farmer and food security. Seed is common carrier of plant pathogens. It carries several destructive pathogens that often take heavy toll causing diseases of crops raised from them. Seed borne diseases are very important from the following points of view; (i) introduction of new pathogens (ii) quantitative and qualitative crop losses and (iii) permanent contamination of soil (Anselme, 1981). Most vital input in crop production programme is seed; it should be of high quality and pathogen free. Pathogen free sound seeds are preferred for sowing to have desired germination, emergence, health seedlings and plant population. Fungi form the largest group among such microorganisms causing seed damage, seed rot diseases at later stages of crop growth till maturity Seed borne fungi may be present in form of hyphae, conidia, oospores, chlamydospores, sclerotia, microspores, hyalospores and phaeospores. Seeds provide natural substrate for the growth of associated fungi, they get associated with seed externally on the seed surface, seed coat and internally with the endosperm, cotyledons, plumule, radical and embryo. Some are on the seed surface as contaminant this influences the seed to plant transmission of the pathogen. Seed borne pathogens result in heavy losses in crop yield and seed quality.

Considering the above facts the present experiment were undertaken with inbreed rice varieties collected from Bangladesh Agricultural Development Corporation, Gabtoli, Dhaka with the following objectives:

1. To find out the prevalence of seed borne fungi from four selected inbreed rice varieties.

2. To isolate different seed borne fungi and to determine their effect on seedlings of four selected inbreed rice varieties of Bangladesh.
3. To determine the effect of identified seed borne fungi on seedling vigority.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Seed borne fungi of rice

Bhuiyan *et al.* (2013) found that a total of seven seed-borne fungi were associated with 40 rice (*Oryzae sativa*) seed samples (cv. BR11 and BRRI dhan28) collected from two upazilas (Narshingdi Sadar and Shibpur) of Narshingdi district in Bangladesh. The identified fungal species were *Bipolaris oryzae,Alternaria padwickii,Sarocladium oryzae,Curvularia lunata,Aspergillus niger*and *Fusarium* spp.

Mansur *et al*. (2013) conducted experiment to detect the fungi associated with the seed samples and to record the germination of seed samples of Parshuram upazila of Feni district,Bangladesh.Three rice were BR6, Pajam and Joya (Local) from Parshuram upazila. The germination of rice seeds of the variety BR6 was 54.67%, while the varieties Joya and Pajam showed 58.00% germination, respectively. Nine seed-borne fungi were detected from these seed samples. The identified fungi were *Fusariumoxysporum, F. moniliforme, Bipolaris oryzae, Alternaria padwickii, Curvularialunata, Aspergillus flavus, Aspergillus niger, Penicillium* spp. and *Nigrospora oryzae.*

Archana and Prakash (2013) performed a survey on 69 rice seed samples collected from different states of India. Totally sixteen genera of fungi *viz. Acremonium, Alternaria, Aspergillus, Bipolaris, Chaetomium, Cladosporium, Curvularia, Exserohilum, Fusarium, Microdochium, Nigrospora, Phoma, Pyricularia, Rhizoctonia, Rhizopus* and *Verticillium* comprising 27 species were found to be associated with the rice seed samples. Among them the most predominant was *Bipolaris oryzae* which is associated with 82.08% seed samples, followed by *Alternaria padwickii* (63.36%). A least incidence of 4.32% was observed with *Bipolaris halodes* and *Acremonium* spp.

Islam *et al*. (2012) examined seeds from ten rice cultivars grown in non saline tidal zones of Patuakhali district to identify seed-borne fungi and their effect on germination. The observed fungi were *Trichoconis padwickii, Curvularia lunata, Fusarium moniliforme, Bipolaris oryzae, Aspergillus flavus, Rhizopus*

sp., *Aspergillus clavatus, Aspergillus niger and Chaetomium* sp.Among the fungi detected, *Trichoconis padwickii* and *Aspergillus flavus were* most predominant.

Ora *et al*.(2011) reportedan important step that seed health testing was to detect seed borne pathogens as in the management of crop diseases. This study was carried out to assess seeds of cultivated hybrid rice varieties (13 imported, two local hybrid rice varieties and two local varieties as check) for seed borne pathogens. Blotter method, paper towel method and agar plate method were used to identify seed borne pathogens and a total of 12 pathogens (*Xanthomonas oryzae, Rhizopus stolonifer, Aspergillus* spp. *Fusarium moniliforme, Phoma* sp. *Bipolaris oryzae, Curvularia lunata, Penicillium* sp. *Alternaria tenuissima, Nigrospora oryzae, Chaetomium globosum* and *Tilletia barclyana*.) were identified. Among these pathogens, *Xanthomonas* spp. 6 *Rhizopus stolonifer, Aspergillus* sp. *Bipolaris oryzae* and *Fusarium moniliforme* are predominant on all tested hybrid rice varieties.

Butt *et al*.(2011) studied seed borne mycoflora of different stored grain of rice varieties by blotter method and its chemical control they reported varieties of rice (*Oryzae sativa* L.) viz. KS-282, Basmati-385, Basmati-370, Basmati Kernal and Basmati-198 were investigated the occurrence of seed-borne mycoflora using blotter paper method and 27%, 19%, 17%, 16% and 14% mycoflora was found associated with the seeds of Basmati kernel, Basmati-385, Basmati- 370, Basmati-198 and KS-282, respectively. Four fungal species namely *Fusariummoniliforme, Alternaria* sp., *Helminthosporium* sp. and *Curvularia* sp. were isolated from different test rice varieties.

Gopalakrishnan *et al*. (2010) conducted an experiment to identify the seed borne pathogens associated with rice seed and recorded 8 genera of fungi viz. *Alternaria,Aspergillus, Bipolaris, Chaetomium, Curvularia, Fusarium, Sarocladium* and *Trichoderma* comprising twelve species. Among them, the most predominant one was *Bipolaris oryzae* (58.89%) followed by *Alternaria padwickii* (52.96%).

Ibiam *et al.* (2008) examined seeds of 3 varieties of rice both in storage and in the field.They isolated *Fusarium moniliforme, Bipolaris oryzae, Fusarium oxysporum,Chaetomium globosum, Curvularia lunata, Aspergillus niger, Aspergillus flavus,Aspergillus terreus, Alternaria tenuis* and *Penicillium* sp. from seeds of three varieties of rice in storage. *Fusarium moniliforme, Bipolaris oryzae, Fusariumoxysporum, Chaetomium globosum, Curvularia lunata* and *Trichoderma harzianum* were isolated from the seeds of the three varieties from the field. *Fusariummoniliforme* was more prevalent than the other fungi.

Haque *et al*. (2007) conducted an experiment during 2003-04 to investigate the health, germination and seedling vigor of the farmers produced rice seeds and to compare the health and quality of seeds produced by trained and untrained farmers. The rice seed samples cv. BR 11 were collected from each of 5 trained and untrained farmers of Babugonj, Barisal to investigate the seed health and quality . Dry inspection, physical sorting, blotter test, germination test and seedling vigor tests were performed. Four fungal genera were associated with the six month stored rice seed samples. The genera were *Aspergillus, Penicillum,Fusarium* and *Curvularia.* In case of freshly harvested rice seed, trained farmers samples yielded the lowest count of *Fusarium* sp. (2.6%), *Bipolaris oryzae* (2.9%), *Curvularia* sp. (0.9%), *Alternaria padwickii* (0.3%), *Nigrospora oryzae* (1.6%).

Tripathi and Dubey (2004) reported that the most destructive seed-borne fungi of rice were*Bipolaris oryzae*, *Pyricularia oryzae, Sarocladium oryzae, Rhizoctonia solani,Sclerotium rolfsii, Fusarium* spp., *Curvularia oryzae* and *Nigrospora oryzae.*

Fakir *et al.* (2003) conducted a detailed investigation to study the effect of different containers and additives on the quality of Boro rice seed. A total number of 16 species of field fungi among the 12 genera viz. *Alternaria padwickii, Alternaria tenuis, Aspergillus niger, Bipolaris oryzae, Cladosporiurn cladosporioides, Curvularia lunata,Curvularia oryzae, Fusarium moniliforme, Fusarium oxysporum, Fusarium semitectum,*

*Microdochium oryzae,Nigrospora oryzae, Pyricularia oryzae, Rhizoctonia solani, Sarocladium oryzae*and *Verticillium* sp.and 10 species of storage fungi viz. *Aspergillus amstelodami, Aspergillus candidus,Aspergillus flavus, Aspergillus nidulans, Aspergillus ochraceus, Aspergillus repens,Aspergillus restrictus, Aspergillus rubber, Aspergillus sydowii and Penicillium* sp.

Nahar (2003) studied status of farmers stored rice seeds of Bogra district and observed the prevalence of*Bipolaris oryzae* (20.36%), *Fusarium moniliforme* (3.76%), *Fusarium oxysporum* (2.88%), *Trichoconis padwickii* (7.61%), *Alternaria alternata* (9.09%) and *Curvularia lunata* (8.51%).

Mew and Gonzales (2002) detected more than 100 fungal species on rice seeds. However, the detection frequency varied considerably. About 20 species of fungal pathogens were detected from rice seed at a time.

Rahman (2002a) collected boro rice seed cv. BRRI from farmer's storage in different containers and tested for determining health status. A total of 11 different fungi viz. *Bipolaris oryzae* (9.75-20.63%), *Alternaria padwickii* (4.13-7.38%), *Fusarium moniliforme* (1.25-8.13%), *Fusarium oxysporum* (1.0-2.63%), *Pyricularia oryzae* (0.13-1.00%), *Rhizopus* spp.(0.13-3.8%), *Curvularia lunata* (4.13-9.5%) and *Alternaria alternata* (4.88-8.63%) were identified from the farmers used rice seed in different containers.

Rahman (2002b) conducted an experiment with farmers saved rice seed cv. Purbachi (local name China) to study the effect of storage container on seed microflora and quality of stored seeds. A total number of 18 different fungal species belonging to 16 genera were associated with the seed sample. Prevalence of common fungal species were detected those were*Curvularia lunata, Trichoconis padwickii,* and *Bipolaris oryzae*.Storage fungi (*Aspergillus* spp,*Penicilliums*pp., *Rhizopus* spp., *Chaetomnium* spp., *Microdochium oryzae, Fusarium solani, Fusarium moniliforme* were also found associated with rice seeds.

Javaid *et al. (*2002) and Khan *et al.* (2000) isolated *Alternaria alternata, A. padwickii, A. longissima, Aspergillus niger, Curvulariaoryzae, C. lunata, Drchslera oryzae, Fusarium miniliforme, F. semitectum, F. oxysporum, F.*

soalni, Pyricularia oryzae, and species of *Phoma, Cercospora, Chaetomium, Sclerotium, Pecicillium, Mirothecium* and *Colletotrichum* from seedsof different varieties of rice collected from different regions of the Pakistan.

Fakir *et al.* (2002) determined five important pathogenic fungi viz. *Alternaria padwickii, Fusarium moniliforme, Bipolaris oryzae, Pyricularia oryzae* and *Sarocladium oryzae* in rice seed samples varied in prevalence with respect to crop season and sites of seed collection.

Naeem Khalid *et al.* (2001) determind the incidence of micro flora, their frequency and impact on germination of four different rice cultivars. They reported five strong fungi viz. *Aspergillus niger, Aspergillus flavus, Penicillium* spp. *Chaetomiumglobosum* and *Rhizopus stolonifer* were associated with rice seeds. The associated microflora reduced the seed germination of all the cultivars.

Singh (2000) evaluated 28 hybrid rice developed at Regional Agricultural Research Station, Titatar and found that*Drechslera oryzae, Curvularia lunata* and *Fusarium* spp.were associated with rice seeds andcomparatively more infection was found with *D. oryzae* followed by *Curvularia lunata* and *Fusarium* spp.In addition to those three fungi, some other fungi, i.e. *Alternaria* spp. *Aspergillus* spp. *Epicoccum* spp. and *Sarocladium oryzae* were also found in the discolored grains. These fungi played an important role which reduced the germination varied from 29 to 80%.

Fakir (2000) reported that rice suffers from more than 60 different diseases. In Bangladesh, 43 diseases are known to occur on the rice. Among these diseases, 27 are seed borne of which 14 are of major importance. He mentioned that major seed-borne diseases were brown spot (*Bipolaris oryzae*), Blast (*Pyricularia oryzae*), sheath rot (*Sarocladium oryzae*), sheath blight (*Rhizoctonia solani*) leaf scaled (*Microdochiumoryzae*), seed rot and seedling blight (*Bipolaris oryzae, Sclerotium rolfsii andFusarium* spp.) and grain spot (*Curvularia lunata, Nigrospora oryzae, Phomaglumarum, Cladosporium* sp.).

Hossain *et al.* (2005) collected seeds of rice varieties R 10, RC 20 and RC 28 from farmers of different villages in the Philippines during the dry season

(January - June) of 1998 to identify the fungi associated with seeds and to evaluate the quality and purity of the seeds. They found that*Fusarium* sp. significantly reducedthe germination of seeds.

Islam *et al.* (2000) conducted an experiment with the nine seed samples of rice cultivar BR11 collected from farmer's storage and analyzed for *B. oryzae* incidence using blotter method. Incidence of *B. oryzae, Trichoconis padwickii, Curvularialunata, Aspergillus* spp. and *Penicillium* spp. ranged from 0.0-64%, 16-48%, 1.2 21%, 0.9-19.5% and 0.0-4%, respectively. The presence of spotted seeds produced low quality seedlings.

Khan *et al.* (2000) found *Fusarium moniliforme* (*Gibberella fujikuroi*), *F. semitectum (F. pallidoroseum), Alternaria padwickii, A. alternata, Curvularia oryzae, Drechslera oryzae* and *Aspergillus niger*from 20 rice seed samples.

Rahman *et al.* (2000) tested the efficacy of seed cleaning method (manual seed sorting and floatation in water) to improve the seed quality in rice cv. BB11. The seed borne fungi were associated with the treated and untreated seeds were *Bipolaris oryzae,Trichoconis padwickii, Curvularia lunata, Nigrospora oryzae, Alternaria tenuis,Aspergillus* spp. and *Penicillium* spp.

Bicca *et al.* (1998) studied health status of rice seed following blotter method. Fungi found associated rice seeds were *Fusarium* spp., *Phoma* sp., *Helminthosporium* sp., *Rhynchosporium* sp., *Alternaria* sp., *Curvularia* sp., *Nigrospora oryzae* [*Khuskia oryzae*], *Cladosporium* sp., *Aspergillus* spp., *Penicillium* sp.and *Epicoccum* sp. *(Sarocladium oryzae)*, false smut (*Ustilaginoidea virens*), kernel smut (*Tilletia barclayana*), scab (*Fusarium graminearum*) and grain discoloration.

Radha jeyalakshmi (1998) reported that a total of 18 fungal species belonging to twelve genera were found to be associated with the rice field seed samples in Tamil Nadu.

Sharma and Viad (1997) studied rice samples were collected from Himachal states of India and showed that the extent of grain discoloration varied between 4.35 to 79.82%. 10 fungi viz. *Alternaria alternata, Aspergillus niger, Curvulariaoryzae, Curvularia lunata (Cochlibolus smiyabeanus), Tilletia*

barclayana (Khuskia oryzae), Pestolatia oryzae, Phylostictia glumarum (Phoma sorghina), Penicillium spp., *Sarocladium oryzae (Magnapothe salvinii),* were detected. *A. alternata* occurredmost commonly followed by *Curvularia lunata.*

Ali and Deka (1996) reported that 10 fungal species from seven fungi *(Curvularia, Dreschslera, Fusarium, Nigrospora, Aspergillus, Penicillium,* and *Chicothcium)* were associated in discoloration grain of 16 rice cultivars. The frequency of occurrence of these fungi varied considerably on different cultivars. The frequency of *Fusariummonilifome* and *Penicillium* were most frequent among the storage fungi after 8-10 months storage.

Ilyas and Javaid (1995) found that out of 46 samples 30 yielded *Fusarium moniliforme (Gibberella fujikuroi),* 45 *Alternaria padwickii,* 7 *Alternaria longissima,* 41 *Dreschslera oryzae,* 2 *Phoma* spp.and 1 each of*Curvularia oryzae* and *Cercospora* spp.

Riaz *et al.* (1995) examined 255 accessions of rice seeds and found most of the Accessions were contaminated with species of 16 fungal genera. *Alternaria* and *Helminthosporium* spp. occurred most frequently and followed by *Curvularia,Fusarium,* and *Aspergillus* spp.

Bhuiyan *et al.* (1994) detected the incidence of *Pyricularia oryzae* in 28 out of 173 seed sample recorded which was 18.0%. The incidence of the fungus was found more in unfilled gains compared to filled grain.

Mirsa *et al.* (1994) screend 144 seed samples collected from 7 different regions of Philipines during dry and wet season of 1988-89 using standard blotter method. A total of 39 fungal species belonging to 30 genera were isolated. The common species excepting *Pyricularia oryzae* and *Nakatia sigmoideum* were evenly distributed during dry season. During wet season distribution of *Dreschslera* sp. and *Microdochiumoryzae* was even. Infection of both apparently healthy and discolored seeds was the highest with *Alternaria padwickii followed* by *Curvularia* sp.

Sharma *et al.* (1992) detected 10 fungal species of fungi from the rice seeds where *Fusarium moniliforme, Gibberella fujikuroi, Curvularia lunata*

Cochliobolus lunatus, Aspergillus flavus and *Rhizopus* spp. were the most common.

Bokhary (1991) reported that the most frequent genera isolated *Curvularia* (5 spp.), *Ulocladium* (5 spp.), *Aspergillus* (4 spp.) *Alternaria* (4 spp.), *Fusarium, Mucor* and *Penicillium* (2 spp.) each. Discolored grain has lower percentage germination than normal grains and had a higher percentage of fungal infection.

Agarwal *et al.* (1990) worked on seed borne diseases and seed health testing of rice and found 20 seed borne diseases of rice (13 fungal, 6 bacterial and 1 caused by a nematode). The fungal diseases were brown spot (*Bipolaris oryzae*), stack burn (*Alternaria padwickii*), leaf scald (*Rhynchasporium oryzae*), bakanae (*Fusarium moniliforme*), sheath blight (*Rhizoctonia solani*), sheath rot.

Vallejos and Mattos (1990) isolated fungal species from milled rice; most frequently occurred were *Aspergillus candidus, A. versicolor, A. fumigates, A. niger, Trichoconis* spp., *Alternaria padwickii, Nigospora oryzae,* and *Penicillium* spp.

Fakir *et al.* (1990) detected seed borne fungal pathogens of rice seed in Bangladesh; these were *Fusarium* spp. *Trichoconis padwickii, Dreschslera oryzae, Phoma* sp. And *Curvularia lunata.*Among these *F. moniliforme* found to be the most common occurring in 58 and 59 seed sample of Pajam and Mala respectively out of 60 samples of each two varieties. As high 55% seed borne infection of the pathogen was detected in Mala. Seed borne infection by *D. oryzae* causing brown spot in rice higher than the normal seed health standard fixed for those pathogens. Average germination of most of the seed samples was below 80.5 which were lower than the national germination standard.

Mian and Fakir (1989) studied on fungi, moisture content and germinability of rough rice grain during storage and observed that the most predominant fungi were *Helminthosporium oryzae, Curvularia lunata, Cladosporium cladosporioides,Aspergillus* spp. and *Trichoconis padwickii.*

Ahmad *et al*.(1989) detected *Fusarium moniliforme, Trichoconis padwickii, Dreschslera oryzae and Curvularia lunata* from rice seed.

Odebunmi and Osikanlu (1989) isolated *Fusarium moniliforme, C. lunata, H. oryzae, Rhynchosporium oryzae* from the six rice seed varieties: IRAT.110, COL.38, C22,TOX494-SLR, DJII-509, and F.H. 109.

Gajapathy and Kalyansundram (1988) studied on distribution of rice seed micro flora within grain with special reference to storage fungi. Storage fungi found to be invading rice and remain mainly husk and outer layer of karnel. The fungi invading the potential layer were mainly *Aspergillus flavus, A. nidulus, A.niger,* to some extent. The more common ones being *A. candidus, A. glacucus* and sometimes *A. versicolor,Penicillium* spp. were less common there.

Jayaweera *et al*. (1988) reported that 17 fungi namely *Bipolaris oryzae, Curvularia pallescens, C. verruculosa, C. eragrostidis, C. afflnis, Pyrenochaeta terrestris, Trichoconis padwickii, Sodaria fimicola, Fusarium* spp. and *Penicillium citreoviride* significantly reduced the germination of rice seed.

Basak and Mridha (1988) studied the seeds of different varieties of Aman rice collected from Chittagong and Chittagong Hill tracts district of Bangladesh. Prevalence of fungi in 44 seed samples tested by the blotter methods varied with cultivar and location. Among those isolates *Rhizopus* spp. had the maximum prevalence in seeds.

Zakeri and Zad (1987) studied the seed borne fungi associated with the abnormalities of rice seedlings and obtained isolates of *Pyricularia oryzae, Drechslera oryzae, Trichoconis (Alternaria) padwickii, Fusarium graminearum (Gibberela oryzae), Nigrospora (Khuskia) oryzae and Alternaria alternate.*

Sharma *et al.* (1987) observed germination reduction was proportional to discolouration severity of the seed. Of 17 fungi isolated from discoloured seeds, *Fusarium moniliforme, Alternaria alternata, Curvularia lunata* and

Trichoconis padwickii were most common recovery rate of *Gibberella fujikuroi* depended on discolouration severity.

Imolehin (1987) found that *Fusarium moniliforme* and *Drechslera oryzae* were the major pathogens that caused devastating seedling disease of rice in the field (*D. oryzae* 12%, *F. moniliforme* 40%). This work was a survey of the incidence of seed-borne fungi of rice associated with three varieties of rice: Faros 12, 15, and 29 popularly cultivated in Afikpo North local government area of Ebony State and isolated *B. oryzae* (*Drechslera oryzae*), *Curvularialunata, Chaetomium* spp., *Trichoderma* spp., *Aspergillus* spp. and *Penicillium* spp. from twenty-two different rice cultivars from South West Nigeria.

Singh and Kang (1987) observed that the major seed borne pathogens of rice were *Helminthosporium oryzae, Fusarium moniliforme, Curvularia lunata, Aspergillusflavus, Alternaria* and *Penicillium* spp.

Aruna and Chaudhary (1986) listed 34 fungi in 23 rice seed samples from different locations. More were detected by blotter method than by deep freeze and agar plate methods.

Ou (1985) reported that the rice is affected by as many as 36 seed-borne diseases of which 31 were caused by fungi.

Ramadoss (1985) observed the discoloration of grains caused by *Drechslera oryzae, Curvularia lunata, Fusarium moniliforme, Alternaria padwickii* and *Sarocladiumoryzae* decreased seed germination by 10% in CO44 and 3% in IR50.

Roy (1983) studied the fungi associated with discolored rice grain were tested together with their percentage incidence. *Curvularia lunata* was the most common 11 (31%) followed by *Fusarium* spp. (13%) and *Chaetomium* (6%). The incidence of discolored grain was 1-12%.

Mia and Mathur (1983) tested 75 seed samples from different parts of the country in the Aus, T. Aman and Boro seasons and observed that more than 90% samples were infected with *Drechslera oryzae* and *Trichoconis padwikii* and the highest infection in individual samples were 88.5% and 63.0% respectively.

Sovae *et al.* (1983) reported the association of *Alternaria tenuis, Cladosporium herbarum, Curvularia lunata, Epicoccum purpurascens, Helminthosporium oryzae, Rhizoctonia solanii, Pyricularia oryzae, Phoma* spp. in rice seed. The averageincidence of these fungi were 12%, 13%, 35%, 28%, 2%, 6%, 6%, 33%, and 1%respectively.

Imolehin (1983) studied on rice seed-borne fungi and their effect on seed germination and reported that seed-borne fungi affected rice seed germination. Fungal pathogens recorded on twenty-two seed samples of rice cultivars from south-western Nigeria included *Drechslera oryzae, Curvularia lunata, Fusarium moniliforme, Penicillium* spp., *Rhizopus* spp., *Chaetomium* spp., *Trichoderma* spp. and *Cladosporum* spp.

Caratelli and Saponaro (1983) in Brazil isolated *Drechslera oryzae, Pyricularia oryzae* and *Alternaria padwickii* from rice seed, among others *Curvularia* spp. were also found in some cases.

Mendoza and Molina (1980) analyzed the seed samples of 10 rice varieties following blotter method of seed health test. They reported that *Drechslera oryzae, Trichoconis padwickii, Fusarium moniliforme, Curvularia oryzae, Curvularia lunata* and *Aspergillus* spp. were associated with the seed and causing 32%, 10%, 5%, 8%,6%, and 2% seedling abnormalities, respectively.

Riberio (1980) examined 79 samples of rice in Brazil, incidence of *Helminthosporium oryzae* was higher in seed sample tested by the filter paper method, indicating it'spresence inside the seeds and its high transmissibility through them, washing andcentrifuging showed the incidence of *Pyricularia oryzae* (26.3), *Cochliobolus miyabeanus* (13.9%), *Curvularia lunata* (44.3%), *Nigospora oryzae* (22.7%), *Fusarium* sp. (12.6%) and *Alternaria* sp. (44.4%).

Ranganathaiah *et al.* (1979) reported that *Pyricularia oryzae* is one of the most serious pathogens of rice in Kamataka. Out of 50 samples tested 12 were found to be infected with this fungus.

Asokhan *et al.* (1979) studied on the influence of seed borne fungi on germination and post emergence mortality of rice (ADT31) and Ragi (Co7) seedling on treatment of seed with spore suspensions of 12 fungi

Helminthosporium sp., *Curvularia* sp. And *Fusarium* sp. was most inhibitory on rice seed germination.

Reddy and Khare (1978) in India, noted 4 fungi in 42 rice seed samples collected from 41 districts, of which *Drechslera oryzae* and *Trichonis padwickii* were associated with 18 samples. Individual sample the highest incidence of these fungi was 32% and 40%, respectively and both were internally seed-borne.

Miah and Fakir (1977) studied the relationship between germinability and associated seed borne fungi of rice. The most predominant fungi in order of prevalence were *Helminthosporium oryzae, Curvularia lunata, Cladosporium cladosporioides, Aspergillus*spp. and *Trichoconis padwickii.*

Zainun and Nik (1977) collected 23 rice varieties from 11 different locations of Malaysia and isolated 33 seed-borne fungi from such seed, commonly encountered pathogens were *Drechslera oryzae*, *Trichonis padwickii*, *Fusarium moniliforme,Pyricularia oryzae* and *Nigospora oryzae.*

Shrestha *et al*. (1977) isolated *Drechslera, Trichonis, Fusarium, Pyricularia, Nigospora, Curvularia, Alternaria, Phoma* and *Cercospora* from rice seed.

Esuroso *et al*. (1975) conducted an experiment over three years on the seed borne fungi of rice in Nigeria following blotter method. It was revealed that *Drechsleraoryzae, Trichonis padwickii* and *Pyricularia oryzae* were seed borne including some other fungi.

Agarwal and Singh (1974) observed 7 fungal species with *Trichoconis padwickii* as the most common one.They also observed varietal differences of seed borne fungi.Seeds of IR 8 had the least infection and the highest infection was recorded on Krishan. Grain discoloration was associated with heavy infection of *C. lunata, F.moniliforme, F. semitectime, T. padwikii and Trichothecium*spp.

Hossain and Fakir (1974) studied on the seed borne microflora of freshly harvested rough rice varieties, which revealed the association of 10 fungal genera. In order of prevalence these were *Fusarium, Nigospora, Curvularia, Alternaria, Aspergillus,Helminthosporium, Penicillium, Rhizopus, Chaetomium*

and *Sordida*. *Curvularia*, the most predominant genus constituted 28.9% of total fungal isolation and 59.5% of the grains yielded this fungus.

Fakir and Ahmed (1974) investigated the association of seed borne micro flora with the freshly harvested rough rice of Tepi-boro, collected from Bangladesh Agricultural University farm during 1970. About 400 bacterial and more than 11000 fungal colonies were isolated from a total of 7000 grains.Different genera of fungi were identified viz. *Fusarium* spp. (3.5%), *Alternaria tenuis* (7.5%), *Aspergillus* spp. (26.7%), *Helminthosporium oryzae* (4.3%), *Penicillium* spp. (0.5%), *Rhizopus nigricans* (0.6%), *Curvularia lunata (*21.7%) *Tilletia barclayana* (19.1%), *Chaetomium* spp. (0.7%), *Zygorhynchus* sp. (0.7%) and *Sordida* spp. (0.1%).

Singh *et al.* (1972) recorded *Alternaria longissima, Curvularia lunata Fusarium* spp., *F. moniliforme, A. oryzae, Sclerotium* sp., *T. padwickii* and *Phoma*sp. from rice seed samples.

Augiero *el al.* (1966) reported germination failure, foot rot of coleoptile and stem rot and seedling blight of rice were attributed by a number of seed – borne fungi. In order of prevalence they were *Fusarium moniliforme, Penicillium* sp., *Trichoconis padwickii* and *Helminthosporium oryzae, Drechslera oryzae* is one of the important pathogens, which cause severe discolouration of rice grains.

Tullis (1936) from USA described the association of *Curvularia lunata* and *Helminthosporium oryzae* with discoloured rice seeds. The relative occurrence of *Helminthosporium, Curvularia* and *Nigrospora* were 62.5%, 18.7% and 18.7%, respectively.

2.2.Effect of seedborne fungi on seedling health

Nazrul *et al*. (2010) reported lack of healthy rice seed is considered as one of the most important constraints to rice production and productivity in Bangladesh. Healthy rice seed production and its use consider pre-requisites for accelerate agricultural growth and this can play a leading role in bringing rural prosperity and economic transformation.

Nahar *et al*. (2004) studied Farmers stored rice seeds of Bogra district and found apparently healthy seed, spotted seeds, deformed seeds, discolored seeds, chaffy grains and insect damaged seeds were recorded by 77.84%, 16.32%, 3.22%, 1.03% and 0.56% , respectively.

Bateman *et al*. (1999) stated that Seed-borne pathogens may cause seed abortion, seed rot, seed necrosis, reduction or elimination of germination capacity as well as seedling damage. Disease at later stages of plant growth by systemic or local infection can take place.

Sisterna *et al.* (1994) isolated *Fusarium semitectum, F. equiseti, F. graminerum, F. oxysporum, Alternaria* sp., *Bipolaris oryzae, Epicoccum* spp., *Curvularia lunata, C. protuberate* and an unidentified species from 9 rice seed samples with black dots,discoloration, chalky spots and other symptoms from two province in Argentinaduring 1988-1989.

Roy (1993) conducted experiment to determine the rice seed discoloration in Assam, discolored grains, followed by *Fusarium* sp. (13%) and *Chaetomium* (6%).

Shahjahan *et al.* (1988) conducted an experiment during Aman season with rice varieties and lines. A total of 23 fungi species were found to be associated with spotted rice grains.

Kim *et al.* (1984) found that the fungus *Monographella albescens* occurred at a frequency of 1-4% in 2 seed samples, among the 21 fungi detected in 26 samples from Chungnan province. Results obtained indicated that *Gerlachina oryzae* was present only in the chaff, endosperm and seed coat but also in the embryo. Seed borne infection caused seed rot seedling blight and brown

discoloration of the coleoptiles, primary and secondary leaf when infected seeds were sown in agar or in soil.

Neergaard (1979) stated that Seed is the most important input for crop production. Pathogen free healthy seed is crying need for desired plant populations and good harvest. Many plant pathogens are seed-borne, which can cause enormous crop losses. Coincidentally, the most important or devastating crop diseases are seed-borne and caused by fungi. It has also been demonstrated that seed-borne fungi are responsible for poor health of seeds in many crops.

Mathur *et al.* (1972) reported about 73.5% of 388 tested rice seed samples from eleven countries were found to be infected with *Alternaria padwickii*. The pathogen caused rotting in seeds, root and coleoptile with ultimate death of young seedlings.

2.3.Effect of seedborne fungi on seedling vigor

Debnath *et al.* (2012) conducted an experiment to determine vigor index of seedling for controlling the seed borne fungal diseases of maize cultivar cv.BARI Bhutta-6 and thus identified *A. niger, A. flavus, Fusarium* spp.,*P. oxalicum, C. lunata* and *R. stolonifer*from maize seed.They observed that Incidence of the seed borne pathogen caused germination failure as well as reduced the seedling vigor which ultimately reduced the yield of maize.

Islam and Borthakur (2012) conducted experiment to evaluate the effect of some dominant seed borne fungi of *Aijung* rice variety on seed germination and seedling vigor. Twenty dominant fungi were found associated with *Aijung* rice seeds. Analysis of seed borne fungi by blotter method and agar plate method showed that species of *Aspergillus, Fusarium, Alternaria* and *Curvularia* were the dominant genera. Seed germination and seedling vigor tests were conducted using seed inoculation, soil inoculation and seed submergence method. Maximum reduction in seed germination and seedling vigor was caused by species of *Fusarium* in seed inoculation method, by species of *Rhizopus* and *Fusarium* in soil inoculation method and by species of *Aspergillus* in seed submergence method. In experiment healthy rice seeds were soaked in 25, 50, 75 and 100% concentration of 7, 14 and 21 days old culture filtratedthe isolated seed borne fungi.

Utobo *et al*. (2011) studied seed borne fungi associated with eight hybrid (H) and three local check (LC) rice varieties and their effects on grain germination and seedling vigor during the 2007 and 2008 harvesting seasons. A total of 9 fungal genera were isolated and identified from the seed samples. Most frequently isolated fungi were *Trichoconis padwickii, Helminthosporium oryzae* and *Fusariummoniliforme* for hybrid and local check rice varieties respectively. Percentage of germination and seedling vigor were found significant ($p<0.05$) from hybrid to local check rice varieties. Maximum numbers of germinated seed at 5, 9 and 14 DAS were recorded from seed samples of hybrid rice varieties and minimum numbers of germinated seeds at 5, 9 and 14 DAS were observed from that of local check rice varieties. Hybrid

rice showed higher vigor in terms of germination, root length, root weight, shoot length, root weight and vigor index when compared to local check rice varieties.

Hampton (1979) conducted an experiment about the the effects of seed-borne and soil-borne *Drechslera sorokiniana*, the conidial state of *Cochliobolus sativa*, on germination, emergence and seedling vigor of barley were examined. Seed-borne inoculums did not significantly affect laboratory germination, glasshouse and field emergence, or seedling vigor, although infected tissue was found on both sub stem internodes and young leaves. Soil-borne inoculums did not affect seedling emergence at 5°C, 15°C, or 20°C, although lesions on sub crown internodes were found. There was a significant reduction in plumule and root growth and dry plant weight for plants grown at 20°C. At 15°C significant reductions in root length and dry plant weight were recorded. At 5°C, a temperature which did not favor the pathogen, no significant effects on seedling vigor were recorded. Plant weight was recorded. At 5°C, a temperature which did not favour the pathogen, no significant effects on seedling vigor were recorded.

Nanda and Choudary (1972) found 19 fungi associated with discoloured seed that reduced germinability and vigor of rice seeds. *Curvularia lunata, (Cochliobolzrs lunatus)* was the most common (37%), followed *by Fusarium* spp. (13%) and *Chaetomium globosum* (60%).

CHAPTER 3

MATERIALS AND METHODS

In this chapter the detail of different materials used and methodology followed during the experimental period are described.

3.1. Experimental Site

The experiment was carried out in the Plant Disease Diagnostic Laboratory and Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.

3.2. Time of Experiment

This study was conducted during the period of July 2013 to June 2014.

3.3. Collection of Seeds

Commonly used rice variety was collected fromBangladesh Agricultural Development Corporation (BADC), Gabtoli, Dhaka. Bangladesh.

3.4 .Variety used

Altogether four inbreed varieties namely BRRI dhan28, BRRI dhan55, BINA Dhan8, NERICA 10 were used in these experiments.

3.5. Determination of prevalence of seed borne fungi

Blotter method was used to determine the occurrence of seed borne fungi associatedin selected rice seeds as per guideline of ISTA, 2001.

3.5.1. Blotter method

The collected seed samples of rice were analyzed for the presence of major seed borne pathogens by blotter method following the international rules for seed testing agency (ISTA, 2001). Four hundred seeds were tested for each variety. Seeds were surface sterilized by 3% chlorax for 30 seconds then washed 3 times with distilled water.Three piece of blotter paper were soaked in sterilized water and placed at the bottom of 9 cm well labeled plastic petri-dishes. 25 seeds were selected at random from each sample and placed in each plastic petri-dish using a pair of forceps, sure that seeds are placed equidistantly with 16 seeds on the outer ring, 8 seeds at inner ring and 1 seed at center (Figure 2). The lid of each Petri-dish was held in place with gummy cello tape.

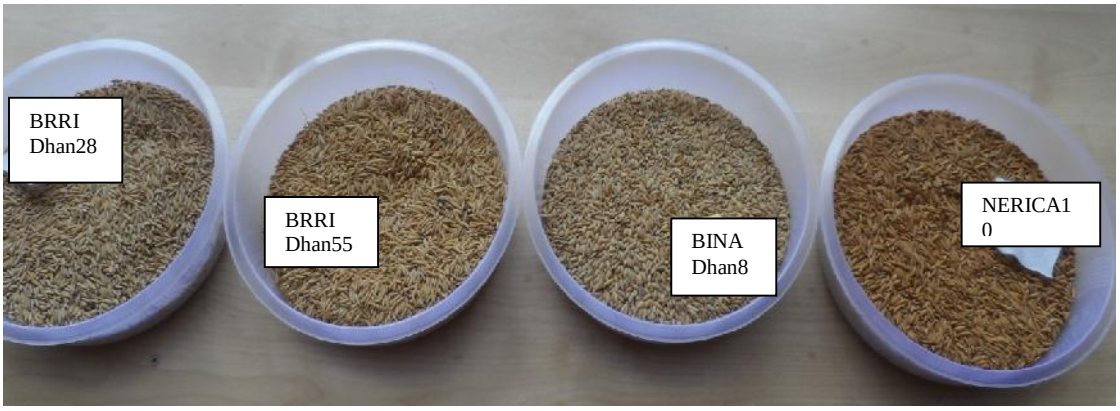


Figure 1. Collected inbreed rice seed sample

Figure 2.Seed health test by blotter method

The petridishes containing seeds were incubated at 20±2°C for 7 days with 12 hours photoperiod.

3.5.2. Inspection of incubated seed samples

After 7-10 days incubation period, individual rice seeds were examined under stereomicroscope in order to record the incidence of different seed borne fungi. With flamed sterilized needles fungal growths on the grains were aseptically mounted in glycerine or lactophanol placed slides and examined under the bionocular compound microscope for fungal diagnostics characteristics. A list of morphological characters of taxonomic important such as spore size, shape, septation, color and their arrangement of the mycelium, density of the colony were compiled for each fungus. Identification of fungus were performed using all the characteristics observed and identification reference manuals of Booth (1971), Barnett and Hunter (1992) and Watanabe (2000). Number of germinated seeds was recorded along with the seed-borne fungi after 7-10 Days of incubation. Most of the associated pathogens were detected by observing their growth characters on the incubated seeds on blotter paper following the keysoutlined by Mathur and Kongsdal(2003).

3.6. Isolation of seed borne fungi from incubated seeds

3.6.1. Preparation of potato dextrose agar (PDA)

PDA medium (appendix-I) was prepared as described by Islam (2009). 200 g peeled and sliced potato was boiled in 500ml water in a bowl for about half an hour. Then the extract of the potato was filtered through cheese cloth. The other two ingredients viz. 20g dextrose and 20g agar were added in the extract and the volume was made up to 1L mark. Then the prepared standard PDA medium was poured in 1000ml conical flask and sterilized (121°C, 15 psi for 15 min) in an autoclave.

3.6.2.Isolation, purification and preservation of seed borne fungal pathogens of rice

Isolation of the seed borne fungi was carried out on PDA medium. PDA plates were inoculated by taking a bit of mycelia from the incubated seed surface and transferred on PDA plates. The fungi were isolated, purified using the hyphal tip culture techniques. Purification was done by reculture. Identification was done following the keys of Barnett and Hunter (1992). The pure cultures were also maintained on PDA slants kept at 5 ^{0}C for further studies.

3.7. Effect of seed borne fungi on seedlings (Water agar test tube method)

This test was done by following methods of Khare *et al.* (1999). In this method, test tube were prepared by pouring 10 ml of 1% water agar in each test tube (2 cm in diameter and 15 cm in length) and then sterilized in an autoclave for about 15 minute under 15 PSI pressure at 121ºC temperature. The water agar in the test tube was solidified at an angle of 60º. 400 seeds for each variety were tested.

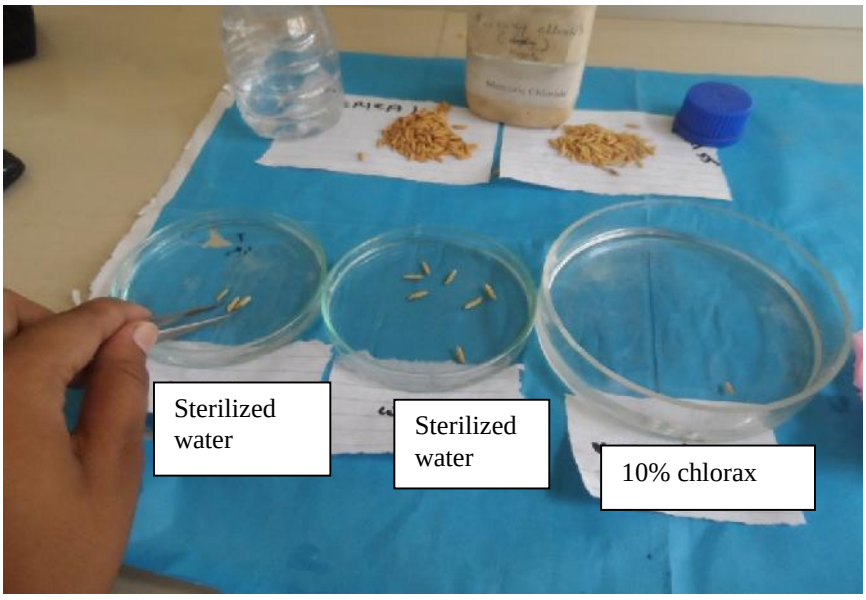


Figure 3.Surface sterilization of seed

Seeds were dipped into 10% chlorax for 1 minute, then wash 3 times with sterilized water (Figure3) and then dried and one seed per test tube were placed on solidified water agar. The tubes were then incubated at erect condition in an air cooled room (temperature 22ºC) under fluorescent day light tube. The

cotton plugs were removed when the seedling reached the rim of the test tube (Figure4).

Data on germination, number of abnormal seedlings, number of diseased seedlings and number dead seeds were recorded.

Figure 4. Seedling symptom test (water agar test tube method)

3.8 Effect of seed borne fungi on seedling vigor (Rolled paper towel method)

Seedling infection and seedling vigor test was done in Rolled Paper Towel method (Warham, 1990). In this method, 400 seeds were randomly taken from each variety and 50 seeds were placed between a pair of moist paper towel. The towels were rolled and the two ends were closed with rubber band as the moist could not remove easily (Figure5). Then the rolled paper towel containing seeds were placed in a15 cmpetridish containing water in an upright position in order to supply required moisture. Then the plates were incubated for 7 days at room temperature under normal 12/12 hours alternating cycle of light and darkness. After 7 days of incubation, observation pertaining to (a) % germination (b) % diseased seedlings (c) % dead seeds (d) root length (e) shoot length and (f) vigor index. For determination of seedling vigor 10 seedlings (normal/ abnormal) were randomly selected from each paper and their individual root and shoot was measured (Figure 6). 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) × % of seed germination.

Figure 5. Seedling vigor test by rolled paper towel method

Figure 6.Ten days old seedlings in rolled paper towel method

3.9. Design of Experiment

The experiment was conducted following Completely Randomized Design (CRD) with four replications. Data collected during experimental period were tabulated and analyzed following Statistical package MSTAT-C. Treatment means were compared with Least Significant Difference Test (LSD) (Gomez and Gomez, 1984).

RESULTS

4.1. Determination of prevalence of seed borne fungi of four selectedinbreed rice varieties by blotter method of seed health testing

4.1.1. Percent seed germination

Germination percentage was recorded of selected rice varieties through blotter papermethods. Significant variations among the varieties in respect of percent seed germination were observed. The highest seed germination percentage was found in BRRI Dhan28 (96.25%) followed by BRRI Dhan55 (94%). The lowest germination 91% was recorded in NERICA 10.These results are presented in table-1.

4.1.2. Incidence of seed borne fungi

Six fungal species of five genera were identified viz. *Aspergillus flavus,Aspergillus niger, Bipolaris oryzae, Fusarium moniliforme, Curvularialunata,Alternaria alternata* (Figure 7-25)

This identification was done by microscopic study.At first fungi were observed under stereoscopic microscope and then cultured in PDA media for above results are presented in Table 1. Results regarding the incidence of *Alternaria alternata* in different varieties of rice ranged from 1.03 to 4.48%.The highest incidence was recorded in NERICA 10(4.48%) followed by BINA Dhan8 (3.59%) and the lowest incidence was observed in BRRI Dhan28 (1.11%).

The incidence of *Aspergillus flavus* ranged from 2.25 to 7.42% where the highest incidence of *Aspergillus flavus* was recorded in BINA Dhan8 (7.42%) followed by BRRI Dhan55 (6.47%).The lowest incidence was recorded in both BRRI Dhan28 and NERICA 10 which was counted2.24%.

The incidence of *Aspergillus niger*ranged from 4.22 to 8.20%.The highest incidence was recorded in NERICA 10 (8.21%) followed by BINA Dhan8 (7.32%).The lowest incidence was recorded in BRRI Dhan28 which was counted 4.22%.

The incidence of *Bipolaris oryzae* ranged from 0.31 to 8.36%.The highest incidence was recorded in NERICA 10 (8.36%) followed by BINA Dhan8

(6.33%).The lowest incidence was recorded in BRRI Dhan28 which was counted 0.31%.

The incidence of *Fusarium moniliforme* was varied from 6.43 to 9.38% where the highest incidence was in NERICA 10 (9.38%) followed by BINA Dhan8 (8.98%).The lowest incidence was recorded in BRRI Dhan28 which was counted 6.43%.

The incidence of *Curvularialunata*was varied from 0.24 to 4.49% where the highest incidence was in NERICA 10 (4.49%) followed by BINA Dhan8 (3.61%).The lowest incidence was recorded in BRRI Dhan28 which was counted 40.24%.

Table 1.Occurrence of seed borne fungi on selected inbreed rice varietiesby blotter method

Inbreedrice varieties	% Pathogen incidence						
	Seed germination(%)	*Alternaria alternata*	*Aspergilus flavus*	*Aspergillus niger*	*Bipolaris oryzae*	*Fusarium moniliforme*	*Curvularia lunata*
BRRI Dhan28	96.25 a	1.03b	2.24c	4.22c	0.31d	6.42c	0.24d
BRRI	94.00b	1.12b	6.47b	6.98b	5.13c	7.42b	1.20 c

Dhan55							
BINA Dhan8	93.00 b	3.58a	7.42a	7.32b	6.33b	8.99a	3.61b
NERICA 10	91.25 c	4.48a	2.24c	8.20a	8.36a	9.38a	4.49a
$LSD_{(0.05)}$	1.37	1.24	0.58	0.72	0.56	0.63	0.35
CV%	0.95	9.84	6.32	6.98	7.23	5.02	9.50

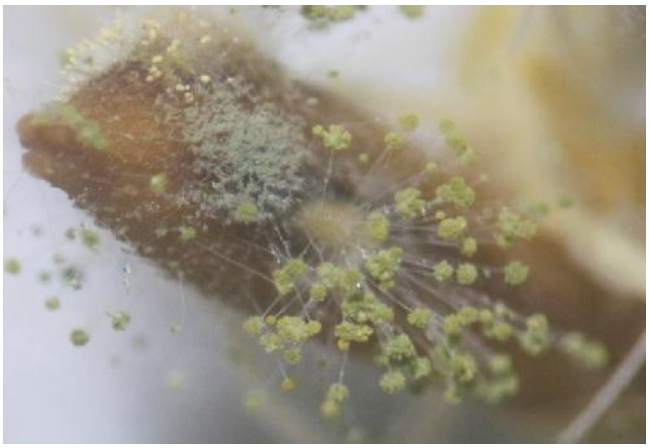


Figure 7.*Aspergillus flavus* on rice seed (under stereo microscope)

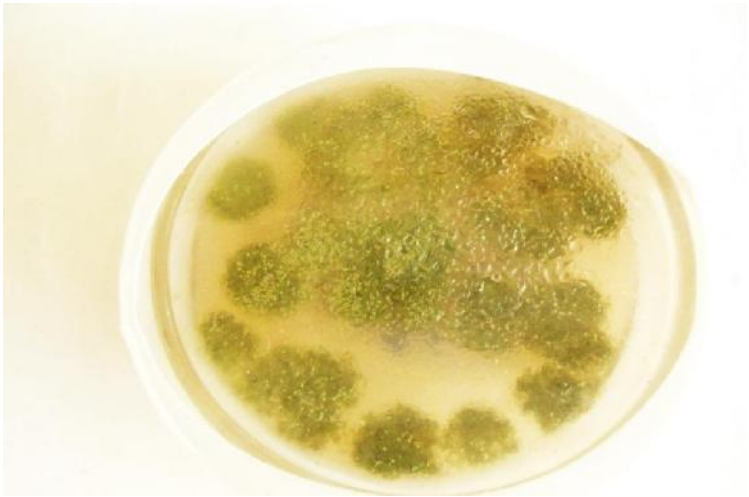


Figure 8. Pure culture of *Aspergillus flavus* on PDA medium

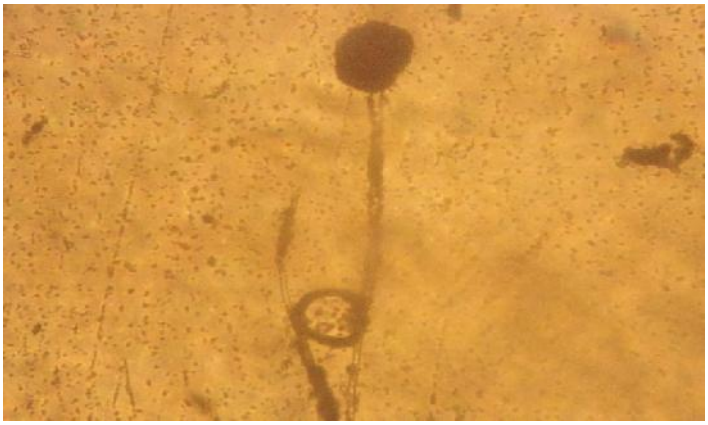


Figure 9.Conidia and conidiophore of *Aspergillus flavus* (under compound microscope at 10 X)

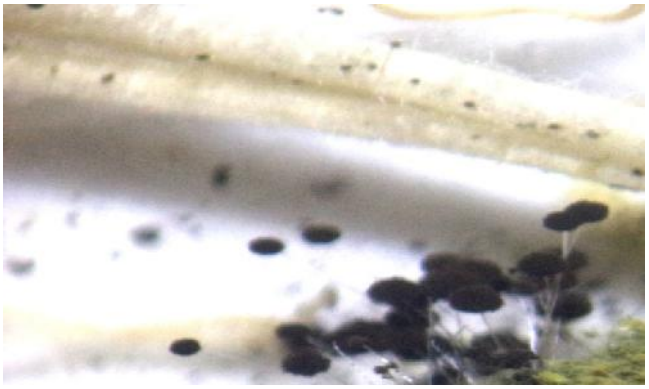


Figure 10.*Aspergillus niger*on rice seed (under stereo microscope)

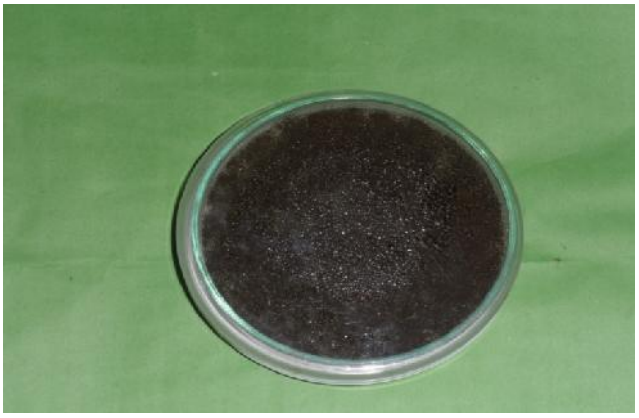


Figure 11. Pure culture of *Aspergillus niger*on PDA medium

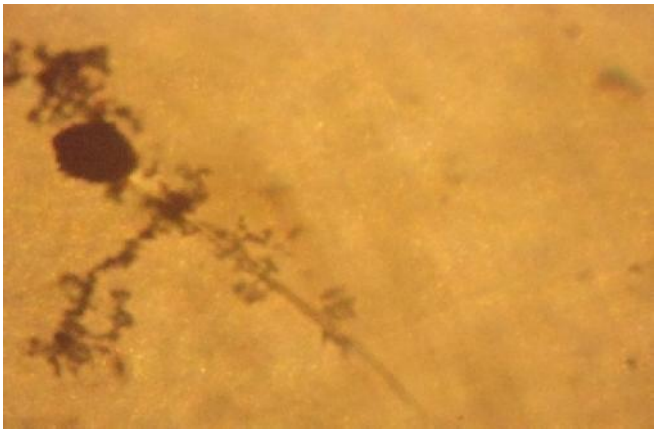


Figure 12. Conidia of *Aspergillus niger*(under compound microscopeat 10 X)

Figure 13.*Fusarium moniliforme* on rice seed (under stereo microscope)

Figure 14. Pure culture of *Fusarium moniliforme* on PDA medium

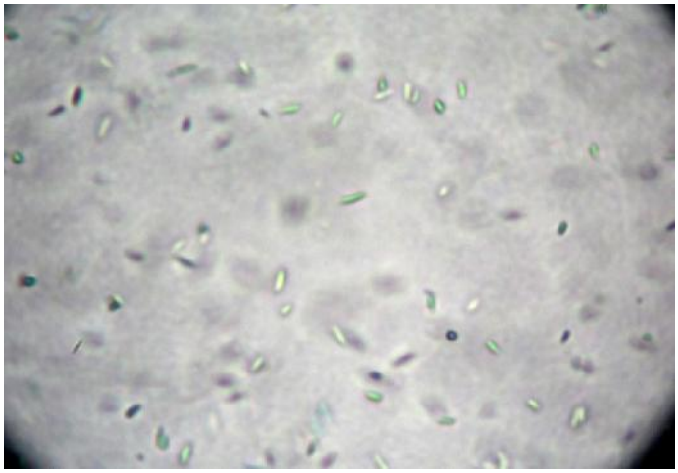


Figure 15. Conidia of *Fusarium moniliforme* (under compound microscope at 10 X)

Figure 16.*Bipolaris oryzae* on rice seed (under stereo microscope)

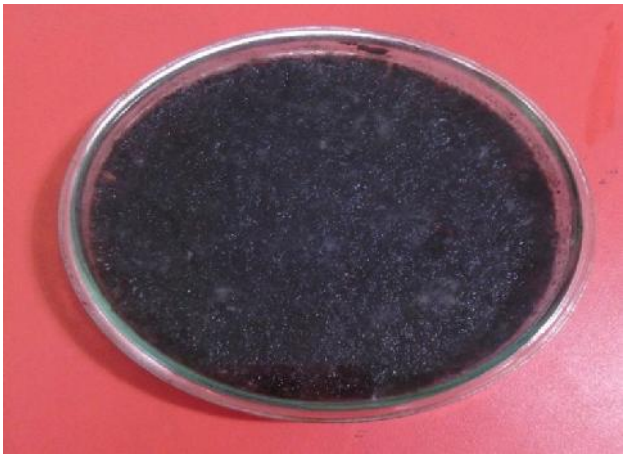


Figure 17. Pure culture of *Bipolaris oryzae* on PDA medium

Figure 18. Conidia of *Bipolaris oryzae* (under compound microscope at 40 X)

Figure 19.Growth of *Alternaria alternata*on rice seed (under stereo microscope)

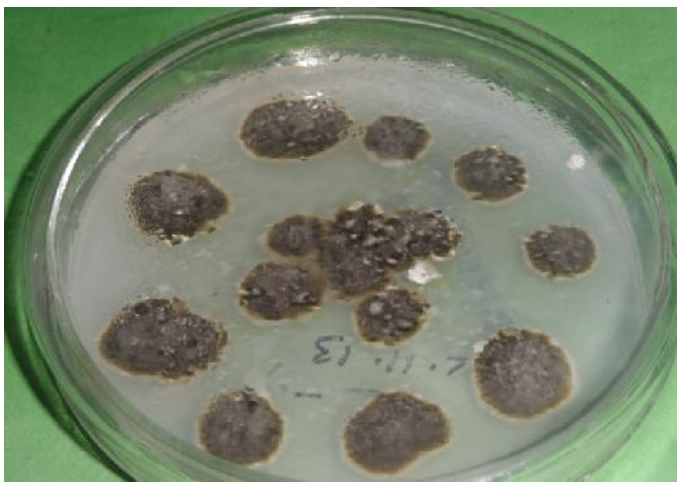


Figure 20. Pure culture of *Alternaria alternata* on PDA medium

Figure 21. Chain of Conidia of*Alternaria alternata*(under compound microscope at 10 X)

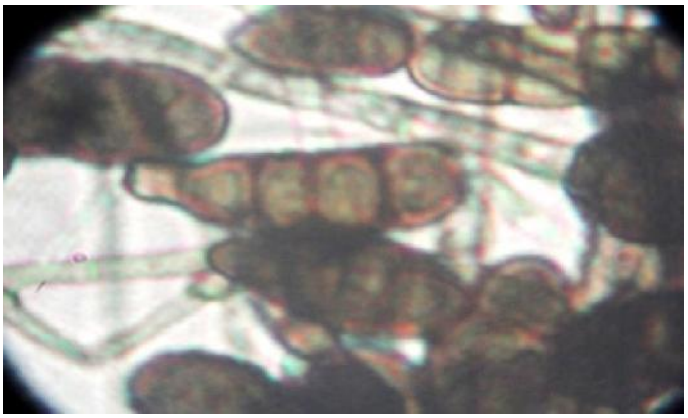


Fig. 22. Conidia *Alternaria alternata* (under compound microscope at 40 X)

Figure 23.*Curvularia lunata*on rice seed (under stereo microscope)

Figure 24. Pure culture of *Curvularia lunata*on PDA medium

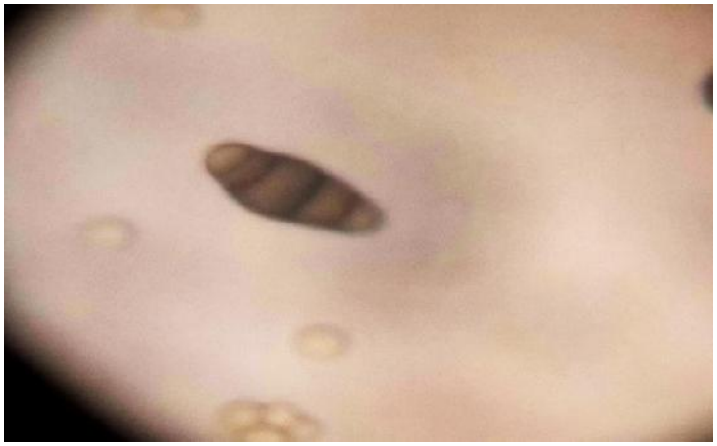


Figure 25.Conidia of *Curvularia lunata*(under compound microscope at 40X)

4.2. Effect of seed borne fungi on seedlings by water agar test tube method

Effect of seed borne fungi on seedlings for selected inbreed rice varieties were determined by water agar method(Table 2). Significant variations were observed among the varieties regarding germination, abnormal seedlings, diseased seedlings and dead seeds (Figure 26-27).

In case of germination, the highest germination was recorded in BRRI Dhan28 (95.25%) followed by BRRI Dhan55 (92.5%) and the lowest was in NERICA 10(90%).

In case of abnormal seedlings, the number of abnormal seedlings ranged from 6.82 to 9.34%.The highest was in NERICA 10 (9.34%) followed by BINA Dhan8 (8.832%) and the lowest was recorded in BRRI Dhan28 (6.82%).

Healthy seedlings varies from 70.16 to 77.93%, where number of healthy seedlings was the highest in BRRI Dhan28 (77.93%) followed by BRRI Dhan55 (75.05%) and the lowest was in NERICA 10(70.16%).

Diseased seedlings varied from 15.25% to 20.52% where the highest percent of diseased seedlings was recorded in NERICA 10(20.52%) followed by BINA Dhan8 (18.73%) and the lowest percent of diseased seedlings was observed in BRRI Dhan28 (15.26%).

Dead seeds ranged from 4.75 to10 %.The highest percent of dead seeds was recorded in NERICA 10(10%) followed by BINA Dhan8 (9.25%) and the lowest percent of dead seeds was observed in BRRI Dhan28 (4.75%). The findings comparisons are presented in figure 27.

Table 2. Effect of seed borne fungi on seedlings of four selected inbreed rice varieties by water agar test tube method

Treatments	% Seed germination	%Abnormal seedlings	%Healthy seedlings	%Diseased seedlings	%Dead Seeds
BRRI Dhan28	95.25 a	6.82c	77.93 a	15.26 c	4.75c

BRRI Dhan55	92.50 b	7.63b	75.04 ab	17.34 bc	7.50b
BINA Dhan8	91.25 bc	8.83a	72.44 bc	18.73 ab	9.25a
NERICA 10	90.00 c	9.34a	70.16 c	20.52 a	10.00 a
LSD(0.05)	1.57	0.57	3.25	2.85	1.57
CV%	1.11	4.53	2.85	10.30	12.96

Figure 26.Seedling symptom test on water ager test tube method.

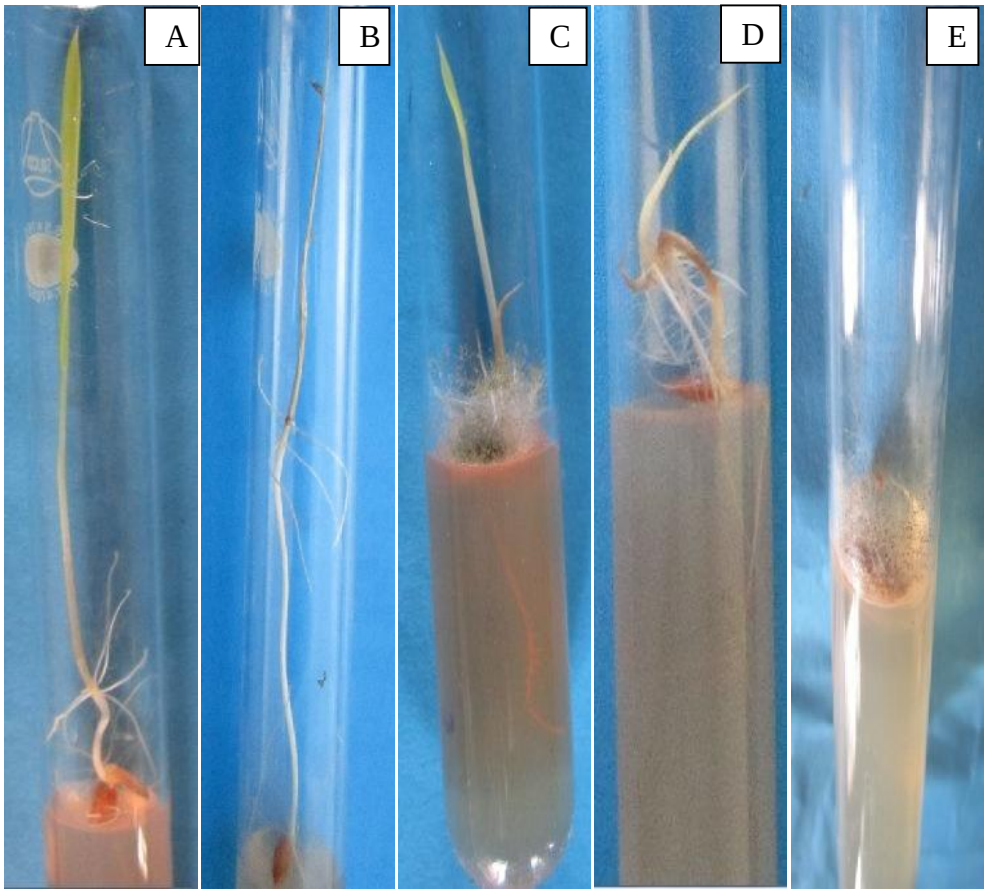


Figure 27.Seedling symptoms on water agar test tube method.

A. Normal seeding
B. Diseased seedling
C. Seedling with fungus
D. Abnormal seedling
E. Dead seed with seed borne fungus

4.3. Effect of seed borne fungi on seedlingsvigor by rolled paper towel method

Effect of seed borne pathogens on seedling vigor of four selected rice varieties were determinedby rolled paper towel method(Figure 28) and significant results found regarding germination, normal seedlings, abnormal seedlings, dead seeds, root length, shoot length and vigor index (Table 3). The highest seed germination percentage was recorded in BRRI Dhan28 (93%) and the lowest germination percent was recorded in NERICA 10 (89%).

Percent of abnormal seedlings varied from 18.09 to 24.39% where the highest value was recorded in NERICA 10 (24.39%) and the lowest value was recorded in BRRI Dhan28 (18.09%). In case of dead seeds the highest value was counted in NERICA 10 (10.25%) followed by BINA Dhan8 (10.25%) and

the lowest value was counted from BRRI Dhan28 (6.75%). The highest root length was recorded in BRRI Dhan28 (10.73 cm) which was statistically similar with BRRI Dhan55 (9.45 cm) and the lowest root length was recorded in NERICA 10 (9.10 cm). Shoot length varied from 4.83 to 6.05 cm. The highest shoot length was recorded in BRRI Dhan28 (6.05cm) and the lowest shoot length was recorded in NERICA 10 (4.83 cm). In case of vigor index the maximum vigor index was recorded in BRRI Dhan28 (1556) and the minimum vigor index was observed in NERICA 10 (1240).

Table 3. Effect of seed borne fungi on seedling health and vigor of four selected inbreed rice varieties by rolled paper towel method

Treatments	Seed germination (%)	Normalseedlings (%)	Abnormal seedlings (%)	Dead seeds (%)	Root length (cm)	Shoot length (cm)	Vigor index
BRRI Dhan28	93.00 a	81.91 a	18.09 c	6.75b	10.73 a	6.05a	1556 a
BRRI Dhan55	90.00 b	78.36 b	21.65 b	9.50a	9.45b	5.18b	1320 b
BINA Dhan8	89.00 b	76.98bc	23.02ab	10.25 a	9.30b	5.00bc	1280 bc
NERICA 10	89.00 b	75.61 c	24.39 a	10.25 a	9.10b	4.83c	1240 c
$LSD_{(0.05)}$	1.62	1.57	1.58	1.59	0.39	0.31	64.11
CV%	1.16	1.31	4.71	12.27	2.64	3.82	3.09

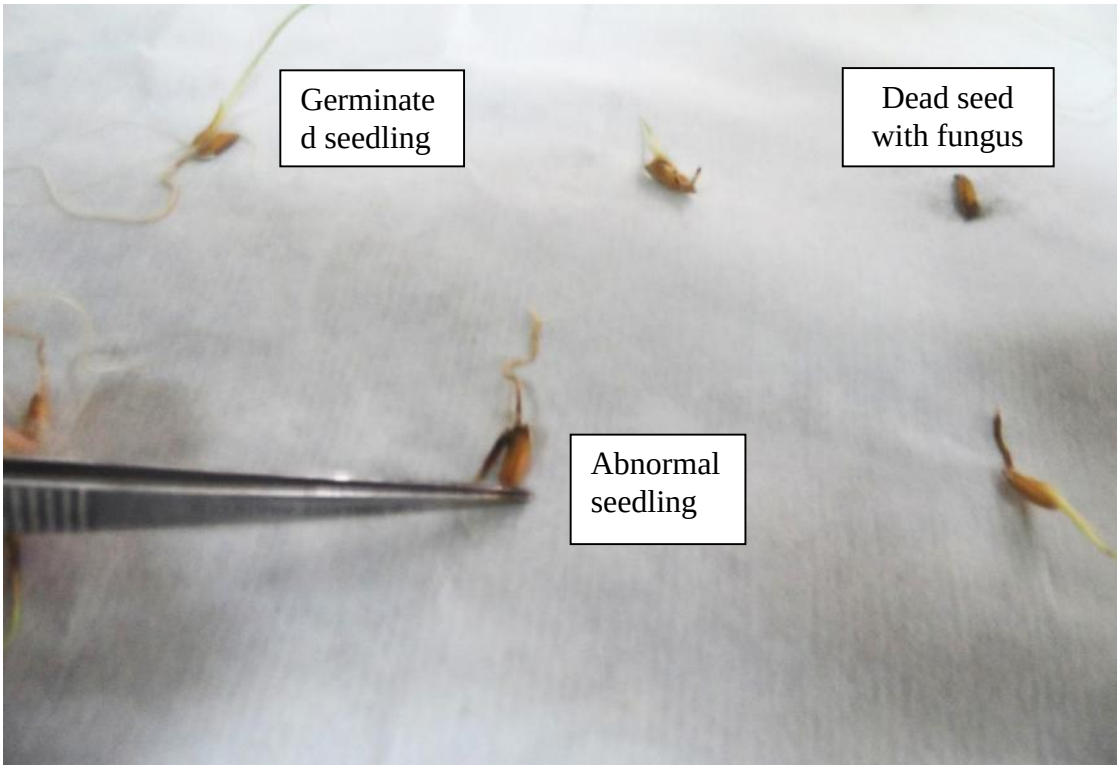
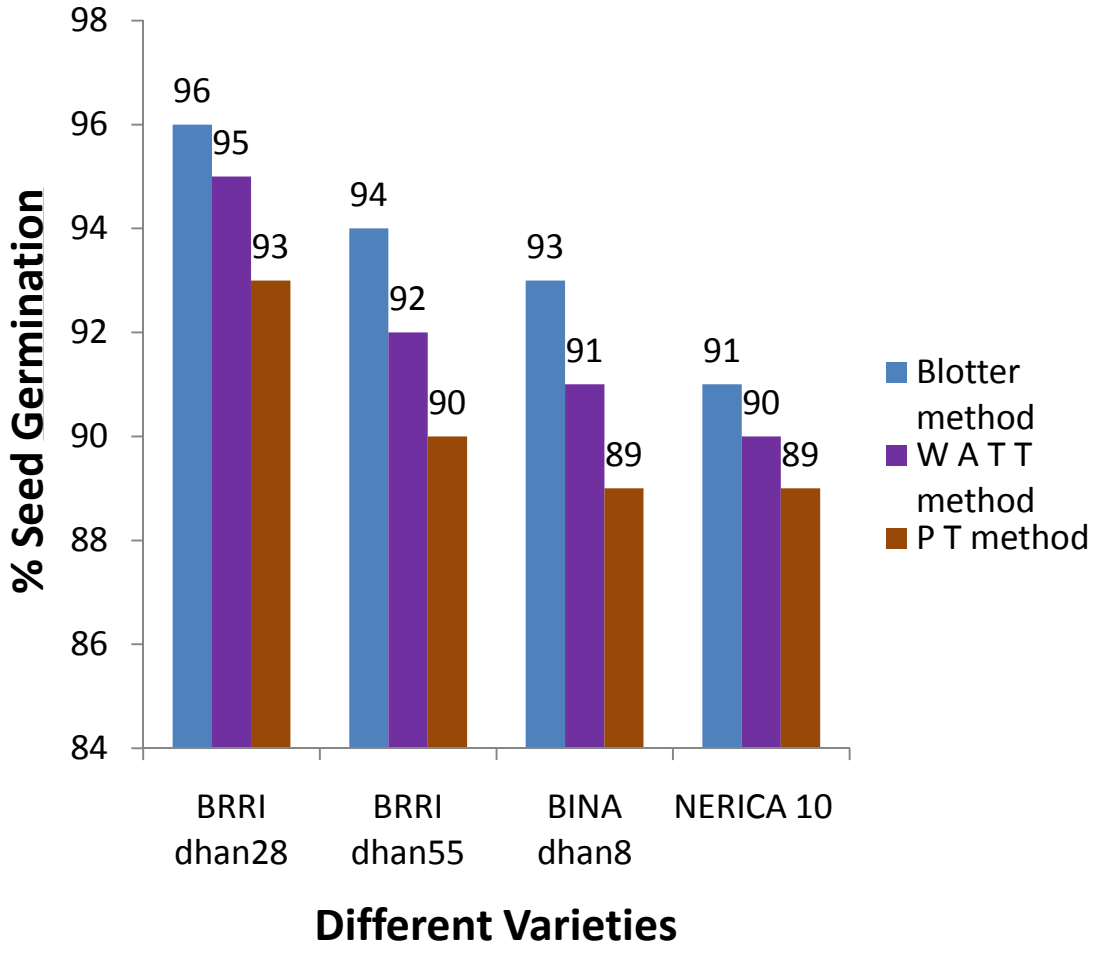


Figure 28.Seedling vigor test by rolled paper towel method.

4.4. Comparative study of germination in different methods

Seed germination (%) did not significantly varied in different methods of incubation. In all cases the maximum germination percent was recorded from the variety BRRI Dhan28 followed by BRRI Dhan55 except in rolled paper towel method (Figure 29).Howeverthe minimum germination percent was recorded from NERICA10 followed by BINA8 except blotter method. In case of blotter method for all varieties % seed germination rate is high followed by water ager test tube method. In paper trowel method % seed germination rate is lower for all varieties.



PT=Paper towel method

WATT=Water ager test tube method

Figure 29. Comparative study of germination (%) in different methods

DISCUSSION

The result of the present study revealed that seed borne fungi were present on most of the cultivated inbreed rice varieties in Bangladesh.Lack of healthy rice

seed is considered as one of the most important constraints to rice production and productivity in Bangladesh.

A considerable amount of seed borne pathogenic fungi were detected by using blotter method, rolled paper towel method, and water agar test tube method. Healthy rice seed production and its use consider pre-requisites for accelerate agricultural growth and this can play a leading role in bringing rural prosperity and economic transformationreportedby (Nazrul *et al.,* 2010).

Six seed borne fungi of five genera were found associated with the collected seed samples as detected by blotter method. These incidence of different pathogens were found to vary individually and independently the varieties of rice seeds. The fungi were *Bipolaris oryzae, Aspergillus flavus, Aspergillus niger, Alternaria alternata,Curvularia lunata*and *Fusarium moniliforme.* From the present study it was observed that presence of seed borne fungi in seed reduced the germination percentage which is supported by many workers (Naeem Khalid *et al.,* 2001, Islam and Borthakur, 2012 and Islam *et al*., 2012).Khan *et al*. (1999) tested the seeds of rice varieties BRRI Dhan11, BRRI Dhan 28, BRRI Dhan 29, and BRRI Dhan 40 and found that *Alternaria padwickii*, *Bipolaris oryzae*, *Curvularia lunata*, *Fusarium oxysporum*, *Aspergillus flavus* and *Penicillium* spp. were associated with the seeds where the highest germination (98.5%) and the lowest incidence of fungi (0.5%) were recorded from BRRI Dhan 28.*Fusarium* is known to invade the seed coat, endosperm and embryo resulting in failure in germination and role of *Fusarium* inhibiting germination has been reported by Utobo *et al.* (2011). The adverse effect of *Aspergillus* on the germination of cereals has been reported by Kanujia and Singh, 1975.Imolehin(1987) found that *Aspergillus* spp. were responsible for production of aflatoxins and deteriorate the quality of rice grain*F. moniliforme* and *B. oryzae* reduced the germinablility of seeds(Bora and Gogoi 1993). A total of seven seed-borne fungi were found associated with 40 rice seed samples and the identified fungal species were *Oryzae sativa Bipolaris oryzae, Alternaria padwickii, Sarocladium oryzae, Curvularia lunata, Aspergillus niger*and *Fusarium* spp. in blotter method (Bhuiyan *et al.,*

2013). Islam *et al*.(2012) examined ten rice cultivars grown in non saline tidal zones of Patuakhali district to identify seed borne fungi and their effect on germination and they found that seed germination is decreased with increasing the seed infection among rice cultivars tested.

The highest incidence of *Aspergillus flavus,Alternaria alternata*, *Bipolaris oryzae* and *Fusariummoniliforme* were detected from NERICA 10, BINA Dhan8, BRRI Dhan28 and BRRI Dhan55, respectively. Similar results were obtained by some other researchers (Archana and Prakash, 2013; Ora *et al*., 2011 and Gopalakrishnan *et al*., 2010). Gopalakrishnan *et al*.(2010) conducted experiment to identify the seed borne pathogen associated with rice seed and recorded 8 genera of fungi viz. *Alternaria, Aspergillus, Bipolaris,Chaetomium, Curvularia, Fusarium, Sarocladium* and *Trichoderma* comprising twelve species.They found that the most predominant one was *Bipolaris oryzae* 58.89% followed by *Alternaria padwickii* (52.96%).

In water agar test tube method BRRI Dhan28 was found most superior in terms of all parameters recorded, which gave the highest germination (95%), minimum number of abnormal seedlings (6.84%), minimum number of diseased seedlings (15.84%) and minimum number of dead seeds (4.75%) were recorded from rice variety BRRI Dhan28. NERICA10 showed comparatively poor performance. The findings of the present study partially supported by Guerrero *et al.* (1972) and Islam *et al*. (2000). Guerrero *et al.* (1972) found that most of the seed borne pathogens caused abnormal seedlings and Islam *etal.* (2000) observed that highest lethal seed infection was caused by seed borne pathogens. Rice seeds inoculated with the fungi without fungicide treatment exhibited some pathogenic symptoms such as root rot in NERICA10 varieties. The pathogenic seed-borne fungi include *Aspergillus* spp.and *Fusarium moniliforme*.Such fungi could seriously retard seed germination through softening and necrosis of tissue. This result supported by Mehrotra and Aggarwal(2003).

Germination and seedling vigor of selected inbreed rice varieties showed significant variation. Seeds of BRRI Dhan28 gave maximum germination and

also yielded maximum number of seedling vigor and the highest root length found inBRRI Dhan28. The findings of the present study partially supported by the findings of Islam and Borthakur (2012) and Utobo *et al.* (2011). Islam and Borthakur (2012) reported that *Fusarium moniliforme*, *Rhizopus nigricans* and *Penicillium oxalicum* caused marked reduction in shoot length, where as *Chaetomium herbasum* and *Fusariummoniliforme* caused marked reduction in root length. *Fusarium moniliforme, F.chlamydosporum* and *Aspergillus niger*caused reduction in vigor index. The present study showed that inbreed rice seeds were infected by harmful pathogens and the pathogens were capable to cause diseases of rice and that may cause a great yield loss.The effect of germination and seedling vigor significantly differed from cultivar to cultivar. The lowest percentage of vigor germination was recorded in NERICA10 in which maximum prevalence of various fungi were recorded. The highest effect of the fungal prevalence reflected the lowest germination as recorded. The fungi species were *Aspergillus niger, Curvularia lunata, Bipolaris oryzae and Alternaria alternata*. The present findings of the seed borne fungal organisms were in agreement with the information of seed borne nature of the pathogen reported by Marley and Gbenga (2004).

Seed is one of the most important inputs of technologies for crop production. For successful crop production there is no other alternative but good seed, this is true for all crops including rice. The importance of rice in Bangladesh could never be over emphasized. It provides 92% of the food requirement of the country and 75% of the total requirement. The food demand of this country is increasing but land is decreasing. Therefore, to feed the extra millions in future rice production unit area needs to be increased (Bhuiyan *et al*., 2002). Among the options to increase rice production use of good seed is most important. In Philippines yield increase due to use of good quality seed was 7-25% (Diaz *et. al*., 2001). Different species of seed-borne fungi have been detected from almost all rice growing countries of the world (Singh, 2000). Considerable work has been carried out on the association of fungi with grain spotting (Aguiero *et. al.* 1966, Mia and Mathur, 1983). Grain discoloration caused

coleoptile decay or radicle decay or both (Roy, 1983). Investigation revealed that *Trichoconis padwickii*, one of the seed borne fungi, reduced seed germination and caused rotting of the seed, shoot and coleoptile (Mathur *et. al.*,1972). Rahman *et al. (*2002) investigated the effect of seed discoloration of rice on germination and seedling vigor. They reported that fungi associated with discoloration in rice resulted in loss in viability, germination and seedling vigor. In each case maximum loss in seed viability, germination and seedling vigor were found in seeds with discoloration on embryo and endosperm regions compared to lower categories of discoloration. Though inbreed seeds gave the higher yield, it can be contaminated with different species of fungi. Thus seed treatment or seed health study is advised before sowing. Therefore, this study emphasize that healthy seed is mostly needed prior to rice cultivation.

SUMMARY AND CONCLUSION

The results of the present study revealed that seed borne fungi were present on most of the cultivated rice varieties in Bangladesh. Although in certain instances they occurred in trace and may create the disease in epidemic level. Pathogen free seed is the vital input in agriculture and noticed that a particular pathogen was observed in a particular variety. So the seed health status of rice seed needs to improve for introduction of new pathogen.

The present study was conducted to isolate and identify the seed borne fungi of selected inbreed rice varieties in Bangladesh and to determine their effect on germination and seedling vigor. The research work was carried out in the Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University; Dhaka-1207 during the period of July 2013 to June 2014. The experiment was carried out according to the rules of International Seed Testing Agency (ISTA) with selected inbreed rice varieties namely BRRI Dhan28, BRRI Dhan55, BINA Dhan8 and NERICA10.In blotter method, six fungal species were identified namely *Bipolaris oryzae, Aspergillus flavus, Aspergillus niger, Alternaria alternata,Curvularialunata and Fusarium moniliforme.* The incidence of different seed borne pathogens was found to vary individually and independently among the inbreed varieties.

In blotter method, the highest incidence of *Bipolaris oryzae* (8.35%), *Aspergillus niger*(8.2%), *Alternaria alternata* (4.47%) and *Fusarium* moniliforme (9.38%) were noticed in NERICA10 and*Aspergillus flavus* (7.42%)inBINA Dhan8. BRRI Dhan28 performed best that germinated 96% with lowest percentage of fungal incidence.

In water agar test tube method, the highest seed germination percentage (95%) was found in BRRI Dhan28.NERICA10 variety was found low health quality regardingabnormal seedlings (9.34%), diseased seedlings (20.52%),and deadseeds (10%) compared to other varieties.

In rolled paper towel method, the maximum number of seed germination (93%), highest vigor index (1556) and the minimum number of abnormal seedlings(18.09%) were recorded from BRRI Dhan28. The maximum number

of abnormal seedlings (24.39%), dead seeds (10.25%) and lowest vigor index (1240) were recorded from NERICA10.

High quality seed is not only important for increasing crop production but also proper establishment of sound seed industry in the country. Seed is a common carrier of plant pathogens. Pathogen free seed is the important input material in agriculture. The present experiment showed that many seed borne fungi were associated with rice seeds. Seed borne fungi appeared maybe due to improper management of rice seeds in storage. Considering the over-all findings it was revealed that the seed health status of tested rice seeds is not in a satisfactory level. Farmers are therefore advised to collect the seeds from reliable sources and rice seed should be treated before sowing.

.

REFERENCES

Agarwal, P.C., Mortensen, C.N. and Mathur, S.B. (1990). Seed Borne Disease and Seed Health Testing of Rice. *Phytopathol*.**30**: 106.

Agarwal, V.K. and Singh, O.V. (1974). Relative percentage incidence of seed borne fungi associated with different varieties of rice seed. *Rev. Pl. Pathol*.**55** (1):38.

Ahmed, M., Hossain, M., Hassan, K.and Dash, C.K. (2013).Efficacy of Different Plant Extract on Reducing Seed Borne Infection and

Increasing Germination of Collected Rice Seed Sample. *Uni. J. Pl. Sci.***1**(3): 66-73.

Ahmed, S.I., Siddiqui, N.V. and Khan, M.Q. (1989). Seed borne fungi associated with seed lots different paddy cultivars in Pakistan. *Pakistan J. Bot*. **21** (2): 309.

Ali, M.S. and Deka, B. (1996).Role of Fungi Causing Grain Discoloration of Rice and Management under Storage.*Indian J. Pl. Pathol*.**26** (1): 79-85.

Anselme, C. (1981). The importance in culture of pathogenic organisms transmitted by seeds.*Seed Sci. Technol*. **9** (3): 689-695.

Archana, B. and Prakash, H.S. (2013). Survey of seed-borne fungi associated with rice seeds in India. *Int. J.Res. Pure Appl. Microbiol*.**3** (1): 25-29.

Aruna, S. and Chaudhary, K.C.B. (1986).Seed microflora of paddy from Manipur state.*Seed Res.***13** (2):42.

Asokhan, A.R., Ramabardan and Emaravarmban, N. (1979). Influence of seed borne fungi on germination and post emergence mortality of rice (ADT31) and Ragi (Co7) seeds. *Indian J. Microbiol.***19** (4): 232-234.

Augiero,V.M., Exconde, O.R. and Quintana, I.J.(1966). Seed-borne fungi of rice and there response to seed treatment.*Rev.Appl. Mycol.* **46**:30-32.

Baki, A.A. and Anderson, J.D. (1972).Physiological and Biological Deterioration of Seeds.In Seed Biology, Vol. II.*Acad. press, New York*.pp. 283-315.

Barnett, H.L., and Hunter, B.B. (1992).Illustrated Genera of Imperfect Fungi. Minneapolis: Burgess publishing Co. p. 241.

Basak, A.B. and Mridha, A.U. (1988). Microflora associated with different varieties of Aman rice collected from Chittagong and Chittagong Hill tracts district of Bangladesh. *Seed Res.***13** (2): 78-89.

Bateman, G.L. and Kwasna, H. (1999).Effects of number of winter wheat crops grown successively on fungal communities on wheat roots.*Appl. Soil Ecol*. **13** (3): 271–282.

BBS (Bangladesh Bureau of Statistics).(2012). Statistical year book of Bangladesh.Ministry of Planning, Government of People's Republic of Bangladesh.

BBS (Bangladesh Bureau of Statistics).(2014). Statistical year book of Bangladesh.Ministry of Planning, Government of People's Republic of Bangladesh.

Bhuiyan, M.R., Rashid, M.M., Khan, M.A., Hoque, M., Nessa, B., Rafi, M.Y. and Latif, M.A. (2013).Eco-friendly Management of Seed Borne fungi for Sustainable Crop Production.*L. Sci. J.* **10**(4):77-85.

Bhuiyan, S.A.M., Begum, M.M., Gani and Mia, M.A.T. (1994).*Pyricularia oryzae* cv. in rice seeds.*Prog. Agric*. **6** (2):93-98.

Bhuiyan, S.I. and Karim, A.N.M.R. (2002). Rice production in Bangladesh an Overview, Bangladesh Rice Research Institute, Joydebpur, Dhaka. pp. 13-17.

Bicca, F.M., Bandet, L. and Zimmer, G.J. (1998). Separation of discoloured seed from rice seed lots using the gravity table and influence on seed health, Revista, *Brasileria de Sementes*, **20** (1):106-111.

Bokhary, H.A. (1991). Seed borne fungi of rice of Saudi Arabia.*Zeitschrift Fiir pflanzenkran kheiten and pflanzenschutz*.**98** (3): 287-292.

Booth, C. (1971). The genus *Fusarium.*CMI, Kew, Surrey, England, p. 238.

Bora, L.C. and Gogoi, R. (1993).Estimation of seed quality deterioration of deep water rice by seed-borne micro-flora in Assam.*Indian J. Mycol. Pl. Pathol.***23** (2): 214–216.

Butt, A.R., Yaseen, S.I. and Javaid, A. (2011).Seed-Borne Mycoflora of Stored Rice Grains and Its Chemical Control.*J. Animal Pl. Sci*. **21** (2):193-196.

Caratelli, A. and Saponaro, A. (1983). Microflora of rice seeds from Maranhao state, Brazil. *Rice Abs.* **8** (1): 112.-114.

Debnath, M., Sultana, A. and Rashid, A. Q. M. B. (2012).Effect of BAU-Biofungicide and Plant Extracts on Seedling Vigor of Maize. *J. Environ. Sci. Nat.Res.***5**(2): 59 – 61.

Diaz, C., Hossain, M., Merca, S. and Mew, T.W. (2001). Seed quality and effect on rice yield findings from farmer participatory experiments in Central Luzon, Philippines. *J. Crop Sci*.**23**(2):111-119.

Esuroso, O. F., Komolafe, C.O and Aluco, M.O. (1975).Seed borne fungi of rice (*Oryza sativa* L.) in Nigeria.*Seed Sci. Technol*.**3**:661-666.

Fakir, G. A. (2000).An annonated list of seed-borne disease in Bangladesh. Seed Pathology Centre, Dept. of Plant Pathology, Bangladesh Agricultural University, Mymensing.

Fakir, G.A. (1982).An annotated list of seed borne diseases in Bangladesh.Agricultural Information Service, Dhaka, Bangladesh.p.15.

Fakir, G.A. and Ahmed, M.U. (1974). Micro flora of freshly harvested rough rice grains of Tepi boro. *Bangladesh Agril. Sci. Abs***. 2**:160.

Fakir, G.A. and Islam, M.R. (1990).Survey on the health status of rice seeds of Sadar Upazilla, Mymensing.*BAURES progress* **.4***:*87-92.

Fakir, G.A., Hossain, I., Ahmed, M.U., Anam, M.K., Islam, M.N. and Rahman, M. (2003). Effect of ash, chalk powder and neem leaf on quality of Boro rice seed stored in gunny bag , motka, plastic drum and tin jar. Reviewand planning Meeting of the Seed Health Improvement, BRRI, Gazipur, Bangladesh.pp.26-28.

Fakir, G.A., Hossain, I., Ahmed, M.U., Asad-ud doula, M. and Alam, M.M. (2002). Quality of farmer's Boro and T. aman rice seeds collected from Bogra, Rajshahi and Rangpur district of Bangladesh. Proceeding: A report for presentation in the review and planning meeting of the Rice seed health Improvement Sub-project held at BRRI Gazipur, Bangladesh.pp.1-16.

Gajapathy, M. K., and Kalyansundram.(1988). Distribution of rice seed micro flora within grain with special reference to storage fungi.*Indian Phytopath*.**39** (2):288-292.

Gomez, K.A. and Gomez, A.A. (1984).Duncan's Multiple Range Test. Statistical procedures for Agricultural Research.2nd Edn.John Wiley and Sons. pp. 207-215.

Gopalakrishnan, G., Kamalakannan, A. and Valluvaparidasan, V. (2010). Survey of Seed-Borne Fungi Associated with Rice Seeds in Tamil Nadu, India. *LibyanAgric. Res. Cent. J. Int.* **1** (5): 307-309.

Guerrero, F.C., Mathur, S.B. and Neergaard, P. (1972).Seed health testing of rice. Seed borne fungi associated with abnormal seedlings of rice. *Proc. Int. SeedTest. Assoc*. **37**: 985-997.

Hampton, J.G. (1979). Effect on barley seed germination, emergence, and seedling vigor.*J. Exp. Agri.**7***: 387-390.

Haque, A.H.M.M., Akon, M.A.H., Islam, M.A., Khalequzzaman,K.M. and Ali, M.A. (2007). Study of Seed Health, Germination and Seedling Vigor of Farmers Produced Rice Seeds. *Int. J. Sust. Crop Prod.* **2**(4): 34-39.

Hoassain, M.M., Khalequzzaman, K.M., Aminuzzaman, F.M., Mollah, M.R.A. and Rahman, G.M.M. (2005). Effect of plant extracts on the incidence of seed-borne fungi of wheat. *J. Agric.Rural Dev*.**3** (1 & 2): 39-43.

Hossain, N. and Fakir, G.A. (1974). Micro flora of rice grains of Bangladesh. Prevalence of microflora associated with the freshly harvested rough grains ofthree Aus varieties of rice. *Bangladesh Agril. Sci. Abs*. **2**:161.

Ibiam, O.F.A. and Umechuruba, C.I. (2008). A survey of seed-borne fungi associated with seeds of rice (*Oryzae sativa* l faro12, 15, and 29) in storage and the field in afikpo north local government area of ebonyi state. *Scientia Africana*.**7** (2):1-4.

Ilyas, M.B. and Javaid, M.S. (1995). Microflora of Basmati 385 Rice Seeds Collected from Gujranwala, Hafizabad, Sheikupura and Sialkot districts. *Pakistan J.Phytopathol*. **7** (1): 50-52.

Imolehin, E.D. (1983). Rice seed-borne fungi and their effect on seed germination. *Pl. Dis*.**67**: 1334-1336.

Imolehin, E.D. (1987). The rice seed multiplication centres in relation to seed-borne pathogens of rice: A case study of Ondo State Rice Multiplication Centers. *Nigerian J. Pl. Prot*. **11**:37-42.

IRRI (International Rice Research Institute). (2006). World Rice Statistics.*Int. Rice Res.Inst*. http://www.irri.org/science/wrs.

Islam, M.S., Jahan, Q.S.A., Bunnarith, K., Viangkum, S. and Merca, S.D. (2000).Evaluation of seed health of some rice varieties under different conditions.*Bot. Bull. Acad. Sin*. **41**: 293-297.

Islam, M.S., Rahman, H., Pervez, Z., Mahmud, M.R and Alam, A. (2012).Studies on seed borne fungi in rice cultivars grown in non saline tidal zones of patuakhali and their effect on seed germination.*Bangladesh Res.Pub.J*. **6** (3):286-290.

Islam, N.F. and Borthakur, S.K. (2012). Screening of mycota associated with *Aijung* rice seed and their effects on seed germination and seedling vigor. *Pl. Pathol.Quarant*.**2** (1): 75–85.

Islam, T. (2009). Population dynamics of *Phomopsis vexans, Sclerotium rolfsii, Fusarium oxysporum* pv. *lycopersici*and *Trichoderma* in the soil of eggplantfield. An M.S. thesis submitted to the Dept. of Plant Pathology, BangladeshAgricultural University, Mymensingh. pp.48-57.

ISTA. (2001). International Rules for Seed Testing. *Seed Sci. Technol*. **24**: 39-42.

Javaid, M.S., Wahid, A., Idrees, A., Gill, M.A. and Saleem, A. (2002). Seed mycoflora studies in rice. *Pakistan. J. Phytopathol*. **14**: 132-134.

Jayaweera, K.P., R.L.C. Wijesundera and Medis, S. A. (1988).Seed-borne fungi of *Oryza sativa*.*Indian Phytopathol*.**41** (3):355-358.

Kanujia, R.R. and Singh, C.S (1975).Studies on certain aspects of seed borne fungi V. Fungi of some starchy seeds.*Phytopathol.notes.* **28**: 299-301.

Khan, N.U. (1999).Studies on Epidemiology, Seed-borne Nature and Management of *Phomopsis*Fruit Rot of Brinjal, MS Thesis, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. pp. 38-68.

Khan, T.Z., Gill, M. A. and Khan, M.G. (2000).Seed borne fungi of rice from central Punjab and their control.*Pakistan J. Phytopathol*. **12**: 12-14.

Khare, M.N. (1999). Seed health care in seed quality control.*Indian Phytopathol*.**52** (3): 305.

Kim, W.G., Park, J.S. and Yu, S.H. (1984).Seed borne infection and damage to rice seeds and seedlings by seed borne *Gerlachia oryzae*.*Korian J. Plant Protc*.**23** (2):126-131.

Mansur, A., Hossain, M., Hassan, K. and Dash, C.K. (2013). Seed health and quality test of three rice varieties for the detection of fungi associated with seed sample. *Uni. J. Pl. Sci.* **1** (2): 37-42.

Marley, P. S. and Gbenga, O. (2004).Fungicide control of Stenocarpella maydis in the Nigerian Savanna.*Arc.Phytopathol.Pl. Protec*. **37** (1): 19-28.

Mathur, S.B. and Kongsdal,O. (2003).Common Laboratory Seed Health Testing Method for DetectingFungi.First edition.International Seed Testing Association, Bassersdorf, Switzerland, p. 425.

Mathur, S.B., Maallya, J.I. and Neergaard P. (1972).Seed-borne infection of *Trichoconis padwickii* in rice, distribution and damage to seeds and seedlings.Proceeding International Seed Testing Association.**37**(22): 803-810.

Mehrotra, R.S. and Aggarwall, A. (2003).Plant pathology.Teta Mc Graw-hill Publishing Company New Delhi.Second Edition.p.254.

Mendoza, A.M. and Molina, R.P. (1980). A study on seed borne fungi associated with rice seed and their effects on rice seedlings. *J. Araneta Res*. **27**(1-4):50-69.

Mew, T.W. and Gonzales.(2002). A hand book of rice seed-borne fungi.IRRI Science Publishers.pp.42-83.

Mia, M.A., and Mathur, S.B. (1983). Study on seed microflora of rice in Bangladesh. *Seed Res.* **11**(2):254-257.

Miah, I.H. and Fakir G.A. (1977).Fungi of stored rough rice grains and their relationship with germinability. Proceeding: Seed Research Conference BARC, Dhaka. pp.21-22.

Mian, I.H. and Fakir, G.A. (1989).Fungi, Moisture Content and Germinability of Rough Rice Grains during Storage.*Seed Res*. **17**: 169-173.

Misra, J.K., Gergon and Mew, T.W. (1994).Occurrence, Distribution and Phenology of Seed Borne Fungi of Rice in Certain Proviences of Philippine.*Pl.Pathol. Bull.* **3** (4): 229-239.

Naeem Khalid, S. A., Anwar, M., Hoque, A., Riaj and Khan, M. A. S. (2001).Seed borne fungi and bacteria of rice and their impact on seed germination.*Pakistan. J.Phytopathol*. **13** (1):75-81.

Nahar, K.(2003). Seed quality and seedling health of farmers' rice of Bogra District. MS thesis, Department of Plant Pathology, Bangladesh Agricultural University , Mymensingh.

Nahar, K., Islam M.M. and Hossain I. (2004). Study on seed seedling health status of farmers saved rice seed C.V. BR 1 from Bogra. *Bangladesh J. Seed Sci*.**8** (1&2) 1-5.

Nanda, J.S. and Choudary, R.C. (1972).Effect of Grain Spotting in an Early Variety of Rice 'Padma' Seedling Characteristics.**21**:7-10.

Nazrul, A.S.M. and Fakhrul, I.S.M. (2010).Factor demand in the healthy rice seed use in Boro and T. aman: A case study of Bangladesh. *Bangladesh J.Agric. Res*.**35**(2): 297-312.

Neergaard, P. (1979).Seed Pathology 1. The Macmillian Press Ltd. pp. 839.

Neergaard, P. (1977). Seed Pathology. The Macmillan Press Ltd., London. P. 1187.

Odebunmi and Osikanlu, Y.K. (1989).Seed-borne pathogens of rice.Survival underexperimental and field conditions.*Nigeria J. Pl. Prot.* **11**:1-8.

Ora, N., A.N. Faruq, M.T. Islam, N. Akhtar and Rahman, M.M. (2011). Detection and Identification of Seed Borne Pathogens from Some Cultivated Hybrid Rice Varieties in Bangladesh. *Middle-East J. Sci. Res.* **10** (4): 482-488.

Ou, S.H. (1985). Rice Diseases.2nd edn. Commonwealth Mycological Institute, Kew, Surrey, England. pp.61-96.

Radha jeyalakshmi, R. (1998).Biological control of seed-borne diseases of rice. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, developed from discolored seeds might serve as India. pp. 36-38.

Rahman,M.M., Rafi. A., Hameed, A., Akhtar, M.A. Farhatullah, Sohail, K., Shahid, M. and Fahim, (2002).Effect of Storage Containers under Farmers Condition on Seed Mycoflora and Quality of Boro Seed, MS Thesis, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. pp. 25-40.

Rahman, A.J.M.K., Islam, M.M. and Mia, M.A.T. (2000). Evaluation of Cleaning Methods to Improve the Quality of Farmer's Saved Rice Seed.*Bangladesh J.Pl. Pathol.***16** (1& 2): 39- 42.

Ramadoss, N. (1985). Studies on the role of fungal pathogens in grain discoloration of rice. M. Sc. (Ag) Thesis. Tamil Nadu Agricultural University, Coimbatore.p. 69.

Ranganathaiah, K.G., Srinivasaiah, S.M. and Gowda, S.S. (1979). Seed borne infection of rice by *Pyricularia oryzae* in Karantaka.*Current Science***.48**:921:952.

Reddy, A.B. and Khare, M.N. (1978).Seed-borne fungi of rice in Madhya Pradesh and their significance.*Indian Pytopathol*.**3**:300-303.

Riaz, Z.T., Mitsueda and Ahmed, Z. (1995). Seed-borne fungi of rice collected from Pakistan. *Plant Genetic Res. Newsl.* **103**:39-40.

Riberio, A.S. (1980). Fungi found on rice seeds in Rio Grando do sul. *Fitpathologia Brasileria*. **5** (1):59-65.

Roy, A.K. (1983). Rice seed discoloration in Assam, India.*Int. Rice Res. Newsl.***8** (5):10.

Shahjahan, A.K.M., Mia, M.A.T. and Miah,S.A. (1988).Rice grain spotting and associated organisms.*Bangladesh J.Pl. Pathol*.**4**(1&2):1-7.

Sharma, H.L., Randhawa, H.S., Kapur, A. and Singh, S. (1987). Seed Discoloration in Rice.Seed Research and Production Unit.**24** (1): 37-41.

Sharma, P.O. and Viad, A. (1997). Microflora of discolored rice grain and it's pathogen potential in Himachal Pradesh. *Himalayan Microbiol.Diversity*. pp.273-281.

Sharma, R.C., Sharma, H.L.and Singh, H. (1992). Effect of incessant rain on seed health and measures to control damage.International Rice Research Newsletter.**17**(2):17.

Shrestha, S.K., Mathur, S.B. and Neergard, P. (1977).Seed borne organisms in some crops in Nepal. *SeedSci. Technol*.**5** (1):111-121.

Singh, D.V., Agarwal V.K.and Singh R.A. (1972). Effect of fungicidal sprays of the quantum of seed borne infection and germination of rice seeds.**9**:103-105.

Singh, K.D. (2000).Mycoflora on discoloured grains of hybrid rice and its effect on germination in Assam.*Ann. Agric. Biol. Res*. **5**(2):155- 157.

Singh, N. and Kang, M.S. (1987). Effect of different level of nitrogen and dates of transplanting on the seed rot germination and seedling mortality of rice. *Pl. Dis. Res.* **2** (2): 125-126.

Sisterna, M. N., Lori, G.A. and Marassi, J.J. (1994). Symptomatalogy and fungi associated with rice grain spotting in cultivar Irga 409. *Revista de la Facultedde Agronomia La Plata.***70**:13-21.

Sovae, J, Azzini, L.E., Villela, O.V. and Galla, P.B. (1983).Selection of irrigated rice cultivars for low incidence of spotted seeds.*Sumnia.Phytopathol*.**9** (3):179- 185.

Tripathi, P. and Dubey, N.K. (2004).Exploitation of natural products as an alternative strategy to control postharvest fungal rotting of fruit and vegetables.*Postharv.Biol. Technol*. **32**:235-245.

Tullis E.C. (1936). Fungi isolated from discoloured rice Kernels. Technical Bulletin 21, U.S. Department of Agriculture.**12**: 540.

Utobo, E.B., Ogbodo, E.N. and Nwogbaga, A.C. (2011). Seedborne Mycoflora Associated with Rice and Their Influence on Growth at Abakaliki, Southeast Agro-Ecology, Nigeria. *Libyan Agric. Res. Cen. J. Intl.* **2** (2): 79-84.

Vallejos, O.V. and Mattos, C.L. (1990). Microflora of Oryza sativa L. and it's relationship with aflatoxin in Lambayeque. *Phytopathologia.***25** (2):54-59.

Warham, E.J. (1990). Effect of *Tilletia indica* Infection on Viability, Germination and Vigor of Wheat Seed.*Pl. Dis*. **74**: 130-135.

Watanabe, T. (2000). Pictprial Atlas of Soil and Seed Fungi: Morphologies of Culture Fungi and key to Species. CRP Press LLC. p.506.

Zainun, W. and Nik, H.W. (1977).A survey on seed-borne fungi of rice in Malaysia.*Malaysian Appl. Biol.***6** (1):67-74.

Zakeri, Z. and Zad, J. (1987). Seed borne fungi associated with some abnormalities of rice seedlings. *Iranian J. Pl. Patho*l.**23**(1-4):1-27.

APPENDICES

APPENDIX I

Composition of Media:

The compositions of the media used in this thesis work are given below: Unless otherwise mentioned all media were autoclaved t 121^0C for 15 minutes at 15 lb pressure.

Potato Dextrose Agar	(PDA)
Peeled Potato	200g
Dextrose	20g
Agar	17g
Water	1000ml

APPENDIX II

List of ANOVA table

Variable 3
Coefficient of Variation = 0.95%

Var. V A R I A B L E No. 3

2	Number	Sum	Average	SD	SE
1	4.00	385.000	96.250	0.96	0.44
2	4.00	376.000	94.000	0.82	0.44
3	4.00	372.000	93.000	0.82	0.44
4	4.00	365.000	91.250	0.96	0.44
Total	16.00	1498.000	93.625	2.03	0.51
Within			0.89		

Variable 4
Coefficient of Variation = 31.44%

Var. V A R I A B L E No. 4

2	Number	Sum	Average	SD	SE
1	4.00	4.110	1.027	1.55	0.40
2	4.00	4.470	1.118	0.16	0.40
3	4.00	14.310	3.578	0.32	0.40
4	4.00	17.910	4.477	0.22	0.40
Total	16.00	40.800	2.550	1.72	0.43
Within			0.80		

Variable 5

Coefficient of Variation = 6.32%

Var. V A R I A B L E No. 5

2	Number	Sum	Average	SD	SE
1	4.00	8.970	2.243	0.41	0.19
2	4.00	25.880	6.470	0.40	0.19
3	4.00	29.680	7.420	0.39	0.19
4	4.00	31.570	7.892	0.31	0.19
Total	16.00	96.100	6.006	2.33	0.58
Within			0.38		

Variable 6

Coefficient of Variation = 6.98%

Var. V A R I A B L E No. 6

2	Number	Sum	Average	SD	SE
1	4.00	16.870	4.217	0.25	0.23
2	4.00	27.920	6.980	0.66	0.23
3	4.00	29.270	7.318	0.48	0.23
4	4.00	32.810	8.202	0.37	0.23
Total	16.00	106.870	6.679	1.59	0.40
Within			0.47		

Variable 7

Coefficient of Variation = 7.23%

Var. V A R I A B L E No. 7

2	Number	Sum	Average	SD	SE
1	4.00	1.230	0.308	0.16	0.18
2	4.00	20.500	5.125	0.22	0.18
3	4.00	25.300	6.325	0.42	0.18
4	4.00	33.420	8.355	0.53	0.18
Total	16.00	80.450	5.028	3.07	0.77
Within			0.36		

Variable 8

Coefficient of Variation = 5.02%

Var. V A R I A B L E No. 8

2	Number	Sum	Average	SD	SE
1	4.00	25.690	6.422	0.48	0.20
2	4.00	29.690	7.423	0.36	0.20
3	4.00	35.990	8.997	0.26	0.20
4	4.00	37.520	9.380	0.47	0.20
Total	16.00	128.890	8.056	1.29	0.32
Within			0.40		

Variable 9

Coefficient of Variation = 9.50%

Var. V A R I A B L E No. 9

2	Number	Sum	Average	SD	SE
1	4.00	0.960	0.240	0.21	0.11
2	4.00	4.810	1.202	0.18	0.11
3	4.00	14.420	3.605	0.17	0.11
4	4.00	17.970	4.493	0.32	0.11
Total	16.00	38.160	2.385	1.80	0.45
Within			0.23		

Variable 3

Coefficient of Variation = 1.11%

Var. V A R I A B L E No. 3

2	Number	Sum	Average	SD	SE
1	4.00	381.000	95.250	0.96	0.51
2	4.00	370.000	92.500	1.29	0.51
3	4.00	365.000	91.250	0.96	0.51
4	4.00	360.000	90.000	0.82	0.51
Total	16.00	1476.000	92.250	2.21	0.55
Within			1.02		

Variable 4

Coefficient of Variation = 2.85%

Var. V A R I A B L E No. 4

2	Number	Sum	Average	SD	SE
1	4.00	311.710	77.927	1.35	1.05

2	4.00	300.140	75.035	1.83	1.05
3	4.00	289.750	72.438	2.21	1.05
4	4.00	280.640	70.160	2.78	1.05
Total	16.00	1182.240	73.890	3.54	0.88
Within			2.11		

Variable 5

Coefficient of Variation = 10.30%

Var. V A R I A B L E No. 5

2	Number	Sum	Average	SD	SE
1	4.00	61.030	15.257	1.18	0.92
2	4.00	69.350	17.337	1.93	0.92
3	4.00	74.920	18.730	1.84	0.92
4	4.00	82.090	20.523	2.27	0.92
Total	16.00	287.390	17.962	2.59	0.65
Within			1.85		

Variable 6

Coefficient of Variation = 4.53%

Var. V A R I A B L E No. 6

2	Number	Sum	Average	SD	SE
1	4.00	27.260	6.815	0.31	0.18
2	4.00	30.510	7.627	0.23	0.18
3	4.00	35.330	8.832	0.41	0.18
4	4.00	37.370	9.343	0.48	0.18
Total	16.00	130.470	8.154	1.08	0.27
Within			0.37		

Variable 7

Coefficient of Variation = 12.96%

Var. V A R I A B L E No. 7

2	Number	Sum	Average	SD	SE
1	4.00	19.000	4.750	0.96	0.51
2	4.00	30.000	7.500	1.29	0.51
3	4.00	37.000	9.250	0.96	0.51
4	4.00	40.000	10.000	0.82	0.51

Total	16.00	126.000	7.875	2.28	0.57
Within			1.02		

Variable 3

Coefficient of Variation = 1.16%

Var. V A R I A B L E No. 3

2	Number	Sum	Average	SD	SE
1	4.00	373.000	93.250	0.96	0.53
2	4.00	362.000	90.500	1.29	0.53
3	4.00	359.000	89.750	0.96	0.53
4	4.00	357.000	89.250	0.96	0.53
Total	16.00	1451.000	90.688	1.85	0.46
Within			1.05		

Variable 4

Coefficient of Variation = 1.31%

Var. V A R I A B L E No. 4

2	Number	Sum	Average	SD	SE
1	4.00	327.640	81.910	1.04	0.51
2	4.00	313.420	78.355	0.96	0.51
3	4.00	307.930	76.982	0.86	0.51
4	4.00	302.430	75.607	1.21	0.51
Total	16.00	1251.420	78.214	2.59	0.65
Within			1.03		

Variable 5

Coefficient of Variation = 4.71%

Var. V A R I A B L E No. 5

2	Number	Sum	Average	SD	SE
1	4.00	72.360	18.090	1.04	0.51
2	4.00	86.580	21.645	0.96	0.51
3	4.00	92.070	23.018	0.86	0.51
4	4.00	97.570	24.392	1.21	0.51
Total	16.00	348.580	21.786	2.59	0.65

Within			1.03		

Variable 6

Coefficient of Variation = 12.27%

Var. V A R I A B L E No. 6

2	Number	Sum	Average	SD	SE
1	4.00	27.000	6.750	0.96	0.56
2	4.00	38.000	9.500	1.29	0.56
3	4.00	41.000	10.250	0.96	0.56
4	4.00	41.000	10.250	1.26	0.56
Total	16.00	147.000	9.188	1.80	0.45
Within			1.13		

Variable 7

Coefficient of Variation = 2.64%

Var. V A R I A B L E No. 7

2	Number	Sum	Average	SD	SE
1	4.00	42.900	10.725	0.17	0.13
2	4.00	37.800	9.450	0.31	0.13
3	4.00	37.200	9.300	0.32	0.13
4	4.00	36.400	9.100	0.18	0.13
Total	16.00	154.300	9.644	0.70	0.17
Within			0.25		

Variable 8
Coefficient of Variation = 3.82%

Var. V A R I A B L E No. 8

2	Number	Sum	Average	SD	SE
1	4.00	24.200	6.050	0.13	0.10
2	4.00	20.700	5.175	0.17	0.10
3	4.00	20.000	5.000	0.27	0.10
4	4.00	19.300	4.825	0.21	0.10
Total	16.00	84.200	5.263	0.52	0.13
Within			0.20		

Variable 9
Coefficient of Variation = 3.09%

Var. V A R I A B L E No. 9

2	Number	Sum	Average	SD	SE
1	4.00	6224.400	1556.100	47.88	20.80
2	4.00	5278.000	1319.500	22.95	20.80
3	4.00	5119.300	1279.825	48.60	20.80
4	4.00	4958.000	1239.500	41.76	20.80
Total	16.00	21579.700	1348.731	132.39	33.10
Within			41.61		