SURVEILLANCE OF SEED BORNE PATHOGENS AND EVALUATION OF HEALTH STATUS OF HYV SEEDS OF RICE GROWN IN BADC FARMS OF BANGLADESH

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This is to certify that thesis entitled ***"Surveillance of Seed Borne Pathogens and Evaluation of Health Status of HYV Seeds of Rice Grown in BADC Farms of Bangladesh*** *submitted to the* ***Faculty of Agriculture,*** *Sher-e-Bangla Agricultural University (SAU), Dhaka in partial fulfillment of the requirements for the degree of* ***MASTER OF SCIENCE (MS) IN PLANT PATHOLOGY*** *embodies the result of a piece of bona fide research work carried out by* ***JANNATUL MOWYA Registration no. 15-06628*** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

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

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DEDICATED
TO
MY BELOVED
PARENTS

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Surveillance of Seed Borne Pathogens and Evaluation of Health Status of HYV Seeds of Rice Grown in BADC Farms of Bangladesh

ABSTRACT

Experiments were conducted to evaluate the health status of eleven selected HYV seeds grown in different BADC farms of Bangladesh. Experiments were performed during the period July 2021 to June 2022 at Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The rice variety were BRRI dhan48, BRRI dhan82, BRRI dhan83, BRRI dhan85, BRRI dhan98, BRRI dhan49, BRRI dhan75, BRRI dhan87, BRRI dhan28, BRRI dhan29, BRRI dhan89. Seed health study was conducted following dry inspection and blotter method of ISTA. In inspection of dry seed method the highest purity percentage was found in BRRI dhan49 (99.98%) whereas the highest inert matter percentage (0.37%) was found in BRRI dhan28.10 fungal pathogen, bacterial ooze and unidentified mycelial growth observed on seeds of HYV rice in blotter seed health test method. The identified fungi were *Bipolaris oryzae*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus* sp, *Curvularia lunata*, *Fusarium oxysporum*, *Fusarium moniliforme*, *Nigrospora oryzae*, *Chaetomium* sp, *Pyricularia oryzae* and *Rhizopus stolonifer*. Pathogen incidence of identified fungi, Bacterial ooze and unidentified mycelial growth of fungi were recorded. Among Aus varieties, BRRI dhan48 and BRRI dhan98 showed the highest (49.25%) and the lowest (28.8%) of seed borne pathogenic infection, respectively. In case of Aman varieties, the highest (58.8%) pathogenic infection was found in BRRI dhan75. However, BRRI dhan49 and BRRI dhan87 showed the same (50.25%) seed borne pathogenic infection. On the other hand, among Boro varieties, BRRI dhan29 and BRRI dhan89 showed the highest (35.5%) and the lowest (17.75%) seed borne pathogenic infection respectively. Considering the frequency and occurrence of seed borne pathogen, *Fusarium* sp., *Aspergillus flavus*, *Bipolaris oryzae* and unidentified white mycelial growth were found predominant seed borne pathogens.

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LIST OF SYMBOLS AND ABBREVIATIONS

Full word	**Abbreviations**
Bangladesh Agricultural Development Corporation	BADC
Bangladesh Agricultural University	BAU
Bangladesh Bureau of Statistics	BBS
Degree Celsius	°C
Etcetera	etc.
Food and Agricultural Organization	FAO
And others	*et al.*
Percentage	%
Sher-e-Bangla Agricultural University	SAU
Videlicet (namely)	viz.

INTRODUCTION

Rice (*oryza sativa*) is known as one of the world's primary food crops especially grown in tropical and sub-tropical region. It belongs to the Poaceae family and is a self-pollinated cereal crop. It grows in more than 100 countries and is used as staple food by 60% of the world population. In Asia, it is considered as the most important source of employment and income for rural people. Asia's unique characteristic environment that is hot and humid climate during long monsoon season provide the most favorable agro-ecological environment for rice cultivation, as well as disease development. More than 90% of the world's rice is farmed in Asia, primarily in China, India, Indonesia, and Bangladesh. Japan, Pakistan, and different Southeast Asian countries also grow some rice, but to a lesser extent. About 75% of Bangladesh's total cultivable land is covered by it and constituted about 85% of total food grain production. (Ahmed *et al*., 2013). In 2020-2021, the total estimated area under rice production was 2,89,13,022 acres and the production was 3,76,07,756 MT (BBS, 2021).

Seed is one of the basic elements for crop production and help to increase agricultural productivity. High quality seed is crucial for healthy seed production in the world as well as for enhanced crop yield. Purity, germination, high production potential, and seed health are the traits that are most crucial for good seed. Seed health is the most crucial key quality. The adage "Health is Wealth" also applies to the health of seeds. The most significant and harmful

diseases that affect our valuable crops are brought by pathogens that are carried in seeds. Whether a seed or seed lot is affected by pathogens or not is referred to as the "seed health." Infected seeds cannot germinate well, and the pathogens from infected seeds can spread to seedlings and plants that can develop in the field, resulting in infection. The most important input in agriculture is pathogen-free seed.

Most of the diseases of rice are seed borne. In Bangladesh, approximately 2.5 million tons of rice worth more than Tk. 1200 million are lost annually due to diseases caused by seed borne pathogens (Fakir *et al*., 2003). The predominant seed-borne fungal pathogens of rice that cause infection and proliferate in the field are *Alternaria padwickii*, *Bipolaris oryzae*, *Curvularia lunata*, *Fusarium moniliforme*, *F. oxysporum*, *Alternaria alternata* and so on (Fakir, 2000). Evidently, there is a need to increase the yield and improve the health and seed quality of the crop by controlling seed-borne fungal pathogens. A total of 153 seed-borne diseases were found in rice, of which 18% are quarantine importance, 65% are native, and 17% are storage pathogens (IRRI, 1987).

Generally, farmers don't check the quality and health of rice seed, but there are so many deadly diseases that can be spread through seeds, and there's a good chance that pathogens will remain inside the seed. There are various reasons for Bangladesh's low rice output, but disease and pests are two main contributors which affect seed quality (Khare, 1999). Nazrul *et al*., (2010) reported that one of the major obstacles to Bangladesh's rice production and productivity is lack of quality rice seed. (Bhuiyan *et al*., 2013) reported that a

total of 40 rice (*Oryza sativa*) seed samples (cv. BR11 and BRRI dhan28) obtained from two upazilas (Narshingdi Sadar and Shibpur) of the Narshingdi district in Bangladesh were associated to seven seed-borne fungus. *Bipolaris oryzae*, *Alternaria padwickii*, *Sarocladium oryzae*, *Curvularia lunata*, *Aspergillus niger*, and *Fusarium* spp. were the identified fungi. It was found that these diseases cause damages at different stages of plant, including storage, seed germination, seedling establishment, vegetative growth, and the reproductive phase.

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). To achieve the desired germination, emergence, healthy seedlings, and plant population, pathogen-free sound seeds are necessary. The endosperm, cotyledons, plumule, radical, and embryo inside the seed, as well as the endosperm and seed coat on the outside, provide as a natural substrate for the growth of seed borne pathogen. Thus pathogens in seeds cause significant reductions in agricultural output and seed quality.

In Bangladesh, high yielding varieties (HYV) of rice play an the important role to achieving food security in cereals. Bangladesh Agricultural Development Corporation (BADC) has the mandate to produce and supply quality seeds of notified crops. (rice, wheat, potato, jute and sugarcane). Currently, the BADC is supplying 33 per cent of the required cereal seeds of rice, wheat and maize. Among the seed supplying organization, BADC supply major portion of

rice seed to the farmers of Bangladesh. HYV rice seed supply is higher by the public sector and hybrid rice seed supply is higher by the private sector (Amin *et al*., 2020).

Considering the above background, this research work is designed to achieve the following objectives:

a) To evaluate the health status of BADC grown HYV seeds;

b) To detect and identify the seed borne pathogens associated with seed samples;

c) To measure the incidence and severity of seed borne pathogens associated with HYV rice seeds grown in different BADC farms of Bangladesh.

CHAPTER 2

REVIEW OF LITERATURE

The primary cereal crop, rice, is affected by a number of seed-borne diseases. In many regions of the world, seed borne fungus and bacteria affected the cultivation of the crop in an economic level. Many researches have been carried out to find seed borne pathogens as rice is the staple food of many countries. Here, an attempt has been made to review the relevant literature available related to the study.

Pain *et al*. (2022) conducted an experiment during the period of July 2018 to January 2019 to determine the effect of seed sources on quality and healthy status in some transplanted Aman rice varieties, at the professor GAF Seed Pathology Center and Laboratory of Department of Seed Science and Technology, Bangladesh Agricultural University (BAU), Mymensingh. Transplanted Aman rice varieties in Bangladesh. They reported that in dry inspection test apparently healthy seeds (0.36g to 5.58g),spotted seeds(9.8g to 17.34g), discolored seeds(0.34 to 3.40g), inert matter (0.00g to 0.24g) and chaffy seeds (0.08g to 1.14g) were recorded (2.75-21.25%), respectively. In seed health test they found seven species of fungi. In seed health test they observed *Alternaria padwickii* ranged from 1.25% to 5.50%, *Aspergillus niger* (0.00 to 1.75%), *Bipolaris oryzae* (0.0% to 1.50%), *Curvularia lunata* (0.00 to 1.00%), *Aspergillus flavus* (0.00% to 1.25%), *Penicillum* sp. (0.00% to 1.50%) respectively.

El-Abbasi *et al*. (2020) conducted an experiment where seed health test including washing test (WT), blotter method (BM), washing after incubation, modified WAI, deep freezing method (DF) and potato dextrose agar (PDA) were applied for detecting seed-borne fungi from rice seeds sample. They stated that *P.oryzae* was recorded only 3 times from rice seed sample along with 1.0 and 1.5 in 2015 using WT and WAI, respectively, and 2.0 in 2016

season using WAI. They also added that Nano-based PCR method could be applied as an appropriate test for rapid detection of rice pathogenic fungus *P.oryzae*.

Hossain *et al*. (2020) stated that *Fusarium moniliforme* is one of the major seed borne pathogen of rice in Bangladesh. He also found that highest 18% of seed infection occured in Boro seeds of Netrokona and lowest 2% was in Gazipur district.

Akter *et al*. (2017) conducted a research for assessing seed health status of four varieties of rice (*Oryza sativa*) viz. BR 11, BRRI Dhan28, BRRI Dhan29 and BRRI Dhan40. Total seven seed borne fungi belonging to six genera viz *Bipolaris oryzae* (1%-6.5%), *Alternaria padwickii* (0.5%-1%), *Curvularia lunata* (1%-5.5%), *Fusarium oxysporum* (2%-9%), *Fusarium moniliforme* (3%-8.5%), *Aspergillus flavus* (0.5%-1.5%) and *Penicillium* spp (0.5%-1%) were found associated with the seeds in blotter method.

Akter and Hossain (2015) tested 15 hybrid rice seed samples and recorded seed samples by dry inspection as apparently healthy seed (35.7 to 85.7%), broken seed (0.4 to 44.3%), spotted seed (0.7 to 8.1%), discolored seed (0.5 to 22.2%), deformed seed (0 to 1.4%), insect damaged seed (0 to 4.9%), broken seed (0.4 to 44.3%), chaffy seed (0 to 1.1%), broken seed (0.4 to 44.3%), and inert matter (0 to 2.6%). In case of purity analysis, pure seed ranged from 92.86 to 100.00% other seeds 0 to 7.14% and inert matter 0 to 2.59%.

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

Archana and Prakash (2013) studied a survey on 69 rice seed samples collected from different states of India. Total sixteen genera of fungi viz. *Acremonium*, *Aspergillus*, *Alternaria*, *Cladosporium*, *Bipolaris*, *Chaetomium*, *Curvularia*, *Exserohilum*, *Phoma*, *Fusarium*, *Microdochium*, *Nigrospora*, *Rhizopus*, *Pyricularia*, *Rhizoctonia*, and *Verticillium* comprising 27 species were found to be associated with the rice seed samples. Among them the most prevalent 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 *Acremonium* spp and *Bipolaris halodes*.

Islam *et al*. (2012) experimented seeds from ten selected rice cultivars grown in non saline tidal zones of Patuakhali district to identify seed-borne microorganism and their effect on germination. The observed fungi were *Fusarium moniliforme*, *Trichoconis padwickii*, *Aspergillus flavus*, *Curvularia lunata*, *Bipolaris oryzae*, *Rhizopus* sp., *Aspergillus niger*, *Aspergillus clavatus*, and *Chaetomium* sp. Among the fungi detected, *Aspergillus flavus* and *Trichoconis padwickii* were most predominant.

Ora *et al*. (2011) stated the seed health testing, an important step was to detect seed borne pathogens as in the process of management of crop diseases. This study was carried out to detect seeds of cultivated hybrid rice varieties (13 imported, two local varieties as check and two local hybrid rice varieties) for seed borne pathogens. Paper towel method, blotter method, and agar plate method were used to identify seed borne pathogens and a total of 12 pathogens (*Aspergillus* spp., *Xanthomonas oryzae*, *Rhizopus stolonifer*, *Phoma* sp.,

Bipolaris oryzae, *Fusarium moniliforme*, *Alternaria tenuissima*, *Curvularia lunata* , *Penicillium* sp., *Nigrospora oryzae*, *Tilletia barclyana* and *Chaetomium globosum*) were identified. Among these pathogens, *Aspergillus* sp, *Xanthomonas* spp. *Rhizopus stolonifer*, *Bipolaris oryzae* and *Fusarium moniliforme* are predominant on all tested hybrid rice varieties.

Butt *et al*. (2011) studied seed borne microorganisms of different stored grain of rice varieties associated with blotter method and its chemical control. They stated varieties of rice (*Oryzae sativa* L.) viz. KS-282, Basmati-370, Basmati-385, Basmati, Kernal and Basmati-198 were investigated the occurrence of seed-borne microorganisms using blotter paper method and 27%, 19%, 17%, 16% and 14% microorganisms were found associated with the seeds of Basmati kernel, Basmati-198 , Basmati385, Basmati- 370, and KS-282, respectively. Four fungal species namely *Alternaria* sp., *Helminthosporium* sp., *Fusarium moniliforme*, and *Curvularia* sp. were isolated from different test rice varieties.

Gopalakrishnan *et al*. (2010) conducted an experiment for the identification of the seed borne pathogens of rice seed and identified 8 genera of fungi viz. *Aspergillus*, *Alternaria*, *Bipolaris*, *Curvularia*, *Sarocladium*, *Chaetomium*, *Fusarium*, 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) worked with 3 varieties of rice both in storage and in the field. They isolated *Bipolaris oryzae*, *Fusarium moniliforme*, *Fusarium oxysporum*, *Chaetomium globosum*, *Aspergillus niger*, *Curvularia lunata*, *Aspergillus flavus*, *Alternaria tenuis*, *Aspergillus terreus*, and *Penicillium* sp. from seeds of three varieties of rice in storage. *Fusarium moniliforme*, *Bipolaris oryzae*, *Chaetomium globosum*, *Fusarium oxysporum*, *Trichoderma harzianum* and *Curvularia lunata* were isolated from the seeds of the three selected varieties from the field. *Fusarium moniliforme* was responsible for highest infection.

Haque *et al*. (2007) conducted an experiment during 2003-2004 to investigate the germination, health and seedling vigor of the farmers produced rice seeds and to compare the quality and health of seeds produced by untrained and trained farmers. The rice seed samples cv. BR 11 were collected 5 trained and untrained farmers of Babugonj, Barisal to investigate the seed quality and health . Dry inspection, physical sorting, blotter test, germination test and seedling vigor tests were performed. Four fungal genera were detected with the six month stored rice seed samples. The genera were *Aspergillus*, *Fusarium*, *Penicillum*, and *Curvularia*. After the process of harvestation trained farmers samples yielded the lowest count of *Fusarium* sp. (2.6%), *Bipolaris oryzae* (2.9%), *Nigrospora oryzae* (1.6%), *Curvularia* sp. (0.9%), *Alternaria padwickii* (0.3%).

Hossain *et al*. (2005) experimented seeds of rice varieties R 10, RC 20 and RC 28 from farmers of different villages in the Philippines in 1998 to identify the fungi associated with seeds and to study the quality and purity of the seeds. They found that *Fusarium* sp. significantly responsible for germination reduction.

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

Fakir *et al.* (2003) experimented an 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*, *Aspergillus niger*, *Alternaria tenuis*, *Bipolaris oryzae*, *Cladosporiurn cladosporioides*, *Curvularia oryzae*, *Curvularia lunata*, *Fusarium moniliforme*, *Microdochium oryzae*, *Fusarium oxysporum*, *Fusarium semitectum*, *Nigrospora oryzae*, *Pyricularia oryzae*, *Rhizoctonia solani*, *Sarocladium oryzae* and *Verticillium* sp.and 10 species of storage fungi viz. *Aspergillus amstelodami*, *Aspergillus ochraceus*, *Aspergillus candidus*,

Aspergillus flavus, *Aspergillus nidulans*, *Aspergillus repens*, *Aspergillus rubber*, *Aspergillus restrictus*, *Aspergillus sydowii* and *Penicillium* sp.

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

Javaid *et al*. (2002) and Khan *et al*. (2000) isolated *Alternaria alternata*, *A. padwickii*, Aspergillus niger, *A. longissima*, *Curvularia oryzae, C. lunata*, *Drchslera oryzae*, *F. semitectum*, *Fusarium miniliforme*, *F. oxysporum*, *F. solani*, *Pyricularia oryzae*, and species of *Phoma*, *Pecicillium*, *Cercospora*, *Sclerotium*, *Chaetomium*, *Mirothecium* and *Colletotrichum* from seeds of different varieties of rice collected from different regions of the Pakistan.

Rahman (2002) 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%), *Fusarium moniliforme* (1.25-8.13%), *Alternaria padwickii* (4.13-7.38%), *Fusarium oxysporum* (1.0-2.63%), *Pyricularia oryzae* (0.13-1.00%), *Rhizopus* spp.(0.13-3.8%), *Fusarium moniliforme* (1.25-8.13%), and *Alternaria alternata* (4.88-8.63%) were identified from the farmers used rice seed in different containers.

Javaid *et al*. (2002) and Khan et al. (2000) isolated *Alternaria alternata*, *A. longissima*, *A. padwickii*, *Aspergillus niger*, *Curvularia oryzae*, *C. lunata*, *Drchslera oryzae*, *F. semitectum*, *Fusarium moniliforme*, *F. oxysporum*, *F. solani*, *Pyricularia oryzae*, and species of *Phoma*, *Cercospora*, *Sclerotium*, *Chaetomium*, *Pecicillium*, *Mirothecium* and *Colletotrichum* from seeds of different varieties of rice collected from different regions of the Pakistan.

Fakir *et al*. (2002) reported five important pathogenic fungi viz. *Alternaria padwickii*, *Bipolaris oryzae*, *Fusarium moniliforme*, *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) experimented the incidence of micro flora, their frequency and impact on germination of four different rice cultivars. They found five strong fungi viz. *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp. *Chaetomium globosum* and *Rhizopus stolonifer* were associated with rice seeds. The associated microflora reduced the seed germination of all the cultivars.

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

Rahman *et al*. (2000) experimented the efficacy of seed cleaning method (floatation in water and manual seed sorting) to improve the seed quality in rice cv. BR11. The seed borne fungi were associated with the treated and untreated seeds were *Curvularia lunata, Bipolaris oryzae, Trichoconis padwickii, Nigrospora oryzae, Aspergillus* spp., *Alternaria tenuis,* and *Penicillium* spp.

Fakir (2000) reported 25 different seed-borne diseases of rice in Bangladesh. Of all the seed-borne diseases, fungi caused 22. Among the seed-borne disease of rice occurring in Bangladesh, 14 were of major importance. He mentioned that the major seed-borne diseases were brown spot (*Bipolaris oryzae*), blast (*Pyricularia oryzae*), sheath rot (*Sarocladium oryzae*), sheath blight (*Rhozoctonia solani*), leaf scald (*Microdochium oryzae*), seed rot and seedling blight (*E. oryzae, Sclerotium rolfsii* and *Fusarium* spp.) and grain spot (*Curvularia lunata, Nigrospora oryzae, Phoma glumarum, Cladosporium* sp.).

Bicca *et al*. (1998) conducted the study with rice seed following blotter method. Fungi observed in rice seeds were *Fusarium* spp., *Phoma* sp., *Rhynchosporium* sp., *Helminthosporium* sp., *Alternaria* sp., *Aspergillus* spp.,

Curvularia sp., *Nigrospora oryzae* (Khuskia oryzae) *Cladosporium* sp., *Penicillium* sp. and *Epicoccum* sp.

Sharma *et al*. (1997) studied rice samples collected from different rice growing locations of Himachal Pradesh. India and showed that the grain discoloration varied between 4.35 to 79.82%. Ten fungi *Aspergillus niger* , *Alternaria alternata, Curvularia lunata* (*Cochliobolus miyabeanus*), *Tilletia barclayana* (*Khuskia oryzae*), *Pestolatia oryzae, Penicillium* sp, *Phyllostictia glumarum* (*Phoma sorghina*) and *Sclerotium oryzae* (*Magnaporthe salvinii*) were detected. *A. alternata* occurred most frequently, followed by *C. lunata*. All fungi, except *Penicillium* sp and *A. niger* were pathogenic.

Ali and Deka (1996) found that ten fungal species from 7 genera (*Curvularia, Fusarium, Drechslera, Nigrospora, Aspergillus, Trichothecium* and *Penicillium*) were associated grain discoloration of 6 rice cultivars. The frequency of occurrence of these fungi varied on different cultivars. The frequency of *F. moniliforme* was highest among the field fungi, while *Penicillium* spp and *Aspergillus* were most frequent among the storage fungi after 8-10 months storage.

Sahu and lena (1995) reported that seed micro flora of 15 semi deep water rice varieties cultivated in 'India was studied by the direct seed inoculation in agar and standard blotter method. In total, 16 fungi from 9 genera and a single bacterium (*Xanthomonas campestris*) were isolated. Among the seed varieties contained the highest percentage incidence associated with fungi and showed the lowest percentage bacteria and fungi and had the highest seed germination rate. Higher levels of microorganism infection were associated with decrease rate of seed germination.

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

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

Bhuiyan *et al*. (1994) recorded 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 prevalence in unfilled gains compared to filled grain.

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

Sharma *et al*. (1992) found 10 fungal species of fungi from the rice seeds where *Fusarium moniliforme, Curvularia lunata, Gibberella fujikuroi, Cochliobolus lunatus, Rhizopus* spp. and *Aspergillus flavus* were the most common.

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

Vallejos and Mattos (1990) observed fungal species in milled rice;most frequently occurred fungi were *Aspergillus flavus, Aspergillus candidas, A.fumigatus, A. versicolor, Alternaria padwickii, A. niger, Penicillium* spp., *Nigrospora oryzae* and *Trichoconis* spp.

Agarwal *et al*. (1990) experimented on seed-borne diseases and seed health testing of rice and recorded 20 seed-borne diseases of rice (13 fungal and 6 bacterial). The disease were stack bum (*Alternaria padwickii*), brown spot (*Bipolaris oryzae*), leaf scald (*Rhynchosporium oryzae*), sheath blight (*Rhizoctonia solani*), bakanae disease and foot rot (*Fusarium moniliforme*/*Gibebrella fujikuroi*), sheath rot (*Sarocladium oryzae*), kernel smut (*Tilletia barclayana*), false smut (*Ustilaginoidea virens*), scab (*Fusarium graminearum* /*Gibberella zeae*), grain discolouration, bacterial leaf blight (*Xanthomonas campestris* pv. *oryzae*), Leaf streak (*X. oryzae*. pv.*oryzicola*), stripe (*Pseudomonas avenae*), sheath brown rot (*P. fuscovaginae*), grain rot (*P. glumae*), and sheath rot (*P. syringae* pv.*syringae*).

Fakir *et al*. (l990) recorded seed borne fungal pathogen of rice seed. In Bangladesh; these were *Drechslera oryzae* , *Curvularia lunata*, *Fusarium* spp, *F. moniliforme, Trichoconis padwickii*, *Phoma* sp. *F. moniliforme* was found to be the most frequent occurring in 58 and 59 seed sample of Pajam and Mala, respectively out of 60 selected sample of each of the two varieties. As high as 55% seed borne infection of the pathogen was recorded in Mala. Seed-borne infection by *D. oryzae* causing brown spot in rice was apparently higher than the national seed health standard fixed for those pathogens. Average germination of the seed samples was below 80%, which are lower than the national germination standard.

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

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

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*.

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

Gajapathy and Kalyansundram (1988) experimented distribution of rice seed micro flora within grain specially storage fungi. All 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 niger, Aspergillus flavus, A. nidulus,* to some extent. The more common ones being *A. glacucus, A. candidus* and sometimes *Penicillium* spp. and *A. versicolor*, were less common there.

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

Singh and Kang (1987) reported that the most prevalence seed-borne pathogens of rice were *Fusarium moniliforme, Curvularia lunata, Helminthosporium oryzae, Aspergillus flavus, Alternaria* and *Penicillium* sp.

Sharma *et al*. (1987) found that incidence of discolouration was higher (23%) in PR 106 rice than in IR8 (19%). Germination failure was proportional to discolouration severity. Among the 17 fungi isolated from discoloured seeds *Fusarium moniliforme, Curvularia lunata, Alternaria alternata,* and *Trichoconis padwickii* were most common.

Ramadoss (1985) reported that discolouration of grains caused by *Alternaria padwickii, Drechslera oryzae, Fusarium moniliforme, , Sarocladium oryzae*

and *Curvularia lunata* decreased seed germination by3% in IR50 and 10% in C044.

Ou (1985) stated that the rice is affected by as many as 36 seed-borne diseases of which fungi are responsible of 31.

Kim *et al*. (1984) stated that the fungus *Monographella albescens* occurred at a frequency of 1-4% in 22 selected seed samples, among the 21 fungi detected in 26 samples from Chungnan province. Results obtained indicated that *Gerlachia oryzae* was present not only in the chaff, endosperm and seed coat, but also in the embryo. Seed borne infection caused seedling blight, seed rot, and brownish.

Sovae *et al*. (1983) found the association of *Curvularia lunata, Alternaria tenuis, Cladosporium herbarum, Helminthosporium oryzae*, *Epicoccum purpurascens, Phoma* spp, *Rhozoctonia solani* and *Pyricularia oryzae* in rice seed. The average incidence of these fungi were 3%, 12%, 13%, 28%, 2%, 6%, 33%,and 1%, respectively.

Imolehin (1987) found that *Drechslera oryzae* and *Fusarium moniliforme* and were the major pathogens that caused devastating seedling disease of rice in the field (*D. oryzae* 12%, *F. moniliforme* 40%).

Mia and Mathur (1983) reported about seed microflora of rice in Bangladesh. They tested seed health of 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 padwickii* and the highest infection in individual samples were 88.5% and 63.0%, respectively. They were also noted the seasonal, local and varietal difference on the incidence of the seed borne fungi.

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

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

Ribeiro (1980) examined 79 samples of rice in Brazil, Incidence of *Helminthosporium oryzae* was higher in sample tested by the filter paper method, indicating its presence inside the seeds and its high transmissibility through them, Washing and centrifuging showed the incidence of *Pyricularia oryzae* (26.3%), *Cochliobolus miyabeanus* (13.9%), *Curvularia lunata* (44.3%), *Nigrospora oryzae* (22.7%), *Fusarium* sp.(12.6%), *Alternaria* sp.(44.4%).

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

Ashokan *et al*. (1979) studied on the influence of seed borne fungi on germination and post emergence mortality of rice (ADT 31) and Ragi (C07) seedlings on treatment of seed with spore suspensions of 12 fungi *Fusarium* sp *Curvularia* sp, *Helminthosporium* sp and were most inhibitory on rice seed germination.

Reddy and Khare (1978) in India, found four fungi in 42 seed samples of rice collected from 41 districts, of which *Trichoconis padwickii* and *Drechslera oryzae* associated with 18 samples. In individual sample the highest incidence of these fungi was 32% and 40% respectively and both were internally as well as externally seed-borne.

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

Miah and Fakir (1977) reported about the relationship between percent of germination and associated seed borne fungi of rice. They found a positive correlation between loss in germinability and increase in storage fungi. They also observed that the most predominant fungi in order of prevalence were *Curvularia lunata*, *Helminthosporium oryzae*, *Cladosporium cladosporioides*, *Trichoconis padwickii* and *Aspergillus* spp.

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

Hossain and Fakir (1974) conducted an experiment on the seed borne microflora of freshly harvested rice varieties, which showed the association of 10 fungal genera. In order of prevalence pathogens were *Curvularia*, *Fusarium*, *Nigospora*, *Helminthosporium*, *Aspergillus*, *Penicillium*, *Chaetomium*, *Rhizopus*, *Alternaria* and *Sordida*. *Curvularia*.

Agarwal and Singh (1974) found 7 fungal species with *Trichoconis padwickii* as the most common one. Highest infection was recorded on Krishna Seeds and IR8 had the least infection. Grain discolouration was associated with heavy infection of *Curvularia lunata*, *Fusarium semitectum*, *Fusarium moniliforme*, *Trichoconis padwickii*, and *Trichothecium* sp.

Solangi *et al*. (1968) from Pakistan reported that the microorganisms associated with the rice varieties. Among these, *Trichoconis padwickii*, *Xanthomonas* spp *Aspergillus* spp. and *Cochliobolus specifier* were predominant.

Augiero *et al*. (1966) stated about germination failure, foot rot of coleoptile, stem rot and seedling blight of rice were attributed by a number of seed borne fungi. In order of prevalence they were *Penicillium* sp, *Fusarium moniliforme*, *Trichoconis padwickii* and *Helminthosporium oryzae*. They also found one of the important pathogens that was *Drechslera oryzae*, which caused severe discolouration of rice grains.

Ibrahim and Farag (1965) reported that *Alternaria tenuis*, *Aspergillus niger*, *A. ustus*, *Fusarium oxysporum*, *Fusarium lateritium*, and *F. solani* were most frequent in apparently healthy seed of four selected rice varieties.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental Site

The experiment was conducted in Seed Health Laboratory and Plant Disease Clinic of the Department of Plant Pathology of Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka-1207**.**

3.2 Experimental Period

The experiment was conducted during the period from July, 2021 to June, 2022.

3.3. Collection of Seeds

Eleven HYV seeds of Rice were collected from Bangladesh Agricultural Development Corporation (BADC), Dhaka, Bangladesh. The collected seed samples were brought directly to Seed Health Laboratory and kept in air tight until use for subsequent studies.

3.4 Variety used

The rice varieties used in this experiment are presented in Table 1.

Table 1. Selected rice seed varieties for the experiment

Season	Variety
Aus season	BRRI dhan48
	BRRI dhan82
	BRRI dhan83
	BRRI dhan85
	BRRI dhan98
Aman season	BRRI dhan49
	BRRI dhan75
	BRRI dhan87
Boro season	BRRI dhan28
	BRRI dhan29
	BRRI dhan89


Aus season varieties

Aman season varieties

Boro season varieties

Figure 1. Selected rice varieties used in this experiment

3.5 Seed Sampling

Seed sampling is the process of obtaining a seed sample to a size suitable for test in which the same constituents are present as in the seed lot and in the same proportions. For seed health testing, seed sampling was done following the rules of ISTA as described the procedure of Mathur and Kongsdal (2003).

3.6 Working samples

Working sample was obtained by following the procedure and methods of Instrumental Rules of ISTA (1999).

3.7 Seed Health Testing

There are many methods for seed health testing. In this experiment, two standard methods were considered viz. inspection of dry seeds and blotter methods for seed health testing as per basic recommendation of ISTA. Those two methods are routinely practiced in all seed health laboratories in the world (Mathur and Kongsdal, 2003).

3.7.1 Inspection of Dry Seeds

The inspection of dry seeds is a method where the presence of fruiting structures of fungi and the effects of fungi on the physical appearance of seeds are examined. The inspection of dry seed is closely associated with the purity analysis as practiced at seed testing station. In the purity analysis the seeds are visually inspected, the sample is divided into three components, i.e. pure seeds, seeds of other crops and inert matter.

3.7.1.1. Working Procedure

For the inspection of dry seeds, 100 g seeds were taken as the working sample depending on their size and weight. The working samples were the same size as the one recommended for “purity analysis” for different crops by the International Rules for Seed Testing (1999). Visual inspection helps to separate the abnormalities, shrinkage, swelling of seeds and fruiting structure observed under microscope. These observations were recorded in the seed health report. Whenever a fruiting structure was observed, that was scrapped from the seed and was examined under compound microscope to identify the fungi (Mathur and Kongsdal, 2003). Seed samples were examined first by naked eye and then observed under a stereomicroscope for confirmation of the below impurities:

Inert matter: It included plant debris, spotted, unfilled & chaffy grains, sclerotia, galls, smut balls, insects etc.

Symptoms: Such as, discoloration, staining, necrosis, malformation and similar indications of infections, including fruiting bodies of fungi, resting hyphae on the seed-surface, spore or bacterial masses on the seed as well as mechanical damage.

3.7.2 Blotter Method

The blotter method is one of the incubation methods where seeds were plated on well water-soaked blotter/filter paper and incubated for 7 days at 22°C temp. under 12 hours alternating cycle of light and darkness. After incubation, fungi has developed on each seed that were examined under different magnification of stereomicroscope and was identified. The identification of the fungi based on the way they grow on seeds is known as growth characteristics and on the morphological characters of fruiting bodies, spores, conidia were observed under a compound microscope. The protocol of ISTA (1985) was followed for blotter method of seed health testing.

3.7.2.1. Working Procedure

A) Preparation of Petri Dishes

Required number of plastic petri dish was collected on a clean working table for plating of seeds. In this method, 9 cm one-time sterilized plastic petri-dish and Whatman no.1 filter paper was used. The number of seeds per petri dish were 10.

To examine 400 seeds from one sample, 40 plastic petri dishes (9 cm) were required. The accession number and date of examination were written on each dish. Three filter papers for each petri dish were counted. Each set of filter paper was dipped into sterilized water and was transferred to the lower dish.

B) Sample Size

At first the whole working sample was poured into a tray and randomly selected for plating by use of sterilized forceps. The seed selection was at random and unbiased. A sample size of 400 seeds was tested for each seed sample as per ISTA (1985) rules.

C) Plating of Seeds

Seeds were placed on wet blotting paper in petri dishes. The pattern of seed plating was followed as ISTA (1985). For rice sample, 10 seeds were placed in each 9 cm petri dish, where 9 seeds were placed in the outer ring, 1 seed were in the center.

Figure 2. Incubation of seeds

D) Incubation of Seeds

All dishes were collected in a tray. Care was taken during handling the dishes in the tray and transferring them to the incubation room so that the plated seeds were not displaced from their original position. The dishes were incubated at 22°C temp. for 7 days in alternating cycle of 12 hours light and 12 hours darkness. The NUV light was used during incubation.

E) Examination of Incubated Seeds After incubation, the petri dishes were brought to the working table in the laboratory. Each seed was examined under stereomicroscope. Examination was conducted in a certain sequence. A line was drawn from the center of the dish to the rim of the dish with the help of a pencil. The first seed to be examined was the one which lies on the right-hand side of the line in the outer circle. Once a thorough examination of the first seed was completed the dish were gently rotted in anti-clockwise direction. Examination of second seed was started when it comes under focus. This procedure was moving from one seed to another. In case of 10 seed in a dish, examination seed in the outer ring was completed first, followed by the seed in the center of the dish. Examination of the habit characters was done for each fungus, the way the fungus grows on the seed under different magnifications of the stereomicroscope. Habit characters were recorded in the note book. For fruiting body of fungi, slides were prepared to examine under compound microscope and identified based on the literatures. Once a fungus was identified to species level under the compound microscope, the infected seed was marked by writing an abbreviation.

Figure 3. Germinating seed and pathogens growing on incubated seeds

F) Observation of Seeds

Each individual incubated seed was observed under stereomicroscope at 40X magnification in order to record the incidence of seed borne fungi. Most of the associated pathogens were detected by observing their growth characters on the incubated seeds on blotter paper following the keys outlined by Mew and Gonzales (2002). For proper identification of fungi temporary slides were prepared from the fungal colony and observed under compound microscope at 100X and 400X. Temporary semi-permanent slides were prepared from the fungal colony and observed under compound microscope.

Figure 4. Observation of seeds and identification of pathogens

G) Recording of Infection

Different fungi in each petri dish were counted by crossing abbreviations one by one. It ensured that all marked abbreviations had been counted. A prescribe working recording sheet was used for data recording. Count of each fungus was recorded immediately after examination of the dish. For each sample, 200 seeds were examined one day and another 200 seeds were examined in another day. The final results of the blotter test were entered in the prescribed seed health report. The following information were recorded in the working sheet during seed examination: colonization of seeds by heavy growth of fungi resulting seed rot and in loss of germination; symptoms in roots e.g. discoloration, rotting; symptoms in cotyledons, coleoptiles, hypocotyls and leaves; death of seedlings and presence and frequency of saprophytes.

H) Identification of Pathogens

Seed borne pathogens of tested rice were identified mainly following the key of Mew and Gonzales (2002). Then slides were prepared from pathogenic structures of seeds and observed under compound microscope and identified with the help of relevant literature and CMI description (Agrios, 2005; Mew and Gonzales, 2002; Mew and Mishra,1994).

3.8 Data Recording

Data were recorded on the following parameters:

3.8.1 Inspection of Dry Seeds

a. % Pure seed

b. % Inert matter

c. % Seeds of other crops

d. % Seeds with fruiting structures of fungi

e. % Seeds with physical abnormalities

3.8.2 Blotter methods

a. % Pathogen infection/incidence

b. % Seed infection

c. Total number of pathogenic infections

d. % Total seed infection by a pathogen

e. % Seed yielding

f. Frequency of pathogen occurrence

3.9 Data Collection Procedure

Pathogen incidence (%) was identified by determining the percentage of no. of infected seed by a specific pathogen to number of examined seeds. The seed infection result has presented as percent incidence for individual pathogen. Disease incidence data were collected by using following formula (Agrios, 2005)

$$\text{Pathogen incidence (\%)} = \frac{\text{Number of infected seed by pathogen}}{\text{Number of examined seeds}} \times 100$$

Total number of seed borne infection was calculated from total number of seed tested for each crop. Total number of seed borne pathogenic infections recorded from the seeds of all varieties of each crop. It means, if 348 seeds were infected by *Fusarium* sp. from 2000 tested seeds of five varieties of rice, then number of pathogenic infection of *Fusarium* sp is 348. Percent of total pathogenic infection was calculated from all seed borne pathogenic infections of a crop. Percentage of pathogenic infection were counted from total number of seed borne pathogenic infection (Sajad *et al*., 2017).

$$\text{\% of total pathogenic infection} = \frac{\text{Number of times pathogen were}}{\text{Total number of pathogenic isolates}} \times 100$$

Percentage of seed yielding of different pathogens was calculated on the basis of total infected seeds. It is the percentage of seed borne infection

from total number infected seeds. It means, 34.32% seeds from total infected 1014 seeds were infected by *Fusarium* sp. Predominant pathogen constitutes at least 5% of total seed borne infection.

CHAPTER 4
RESULTS

Seed borne fungi detected from selected rice varieties are presented in different tables and figures. Prevalence of seed-borne fungal infections varied depending on the seeds of different varieties. Frequency of different pathogen occurrence is also presented with sufficient data in tables from the findings of inspection of dry seeds and blotter method of seed health testing.

4.1 Seed Health Study of HYV rice (Aus) varieties

4.1.1 Health status of HYV rice (Aus) varieties by inspection of dry seed method

A. Physical appearance of seeds

No physical abnormalities were observed in Aus rice varieties (BRRI dhan48, BRRI dhan82, BRRI dhan83, BRRI dhan85, BRRI dhan98) by naked eye. (Table 2)

B. Presence of fruiting structures of fungi

Mycelial structures were found on the seed surface of selected Aus rice variety seeds. Some are visible even with a naked eye. (Table 2).

C. Purity analysis

For purity analysis, 100 gram seeds of each variety were analyzed. The seeds were observed by naked eyes.

4.1.2 Prevalence of seed-borne pathogen of HYV rice (Aus) varieties identified by blotter method

The pathogenic incidence varied significantly among the samples for all pathogens (Table 3).

A. Identified pathogen

Different fungal pathogens viz. *Aspergillus flavus*, *Aspergillus niger*, *Fusarium* sp, *Curvularia lunata*, Unidentified mycelial growth, *Aspergillus* sp., *Nigrospora oryzae*, *Chaetomium* sp, *Trichoderma* sp, Bacterial ooze were observed and recorded. Total 10 microbial species were identified from the seeds of five varieties of HYV varieties of Aus rice. (Table 3).

B. Incidence of seed borne pathogens

In table 3, in case of fungal infection, incidence of *Fusarium* sp was varied from 8.5% to 28.5%. Incidence of mycelial growth of unidentified pathogen was varied from 7% to 22.5%. Incidence of *Curvularia lunata* was varied from 0.5% to 8%. Incidence of *Aspergillus flavus* and *Aspergillus* sp were varied from 1.5% to 17% and 1% to 9.5%. Incidence of *Chaetomium* sp was varied from 1 % to 5.5%. Incidence of Bacterial ooze was varied from 0.5% to 7%. Incidence *Nigrospora oryzae* was varied from 0.5% to 5%. *Trichoderma* sp only found BRRI dhan83 only having 1% incidence (Table 3).

C. Frequency and occurrence of various pathogens recorded in different variety of HYV of rice (Aus) seeds

In table 4, frequency of various pathogen occurrences was calculated in seed samples of different varieties of rice seeds (Aus). It was observed that, the highest 348 seeds from 2000 tested seeds of five varieties of HYV of rice (Aus) were infected by *Fusarium* sp that was 34.32% of total seed borne infection (1014) and 17.4% of the total observed seeds (2000). Moreover, 250 seeds were infected by unidentified mycelial growth that was 24.66% of total seed infection and 12.5% of total seed yield. In case of *Aspergillus flavus* 134 seeds were infected and the frequency of occurrence was 13.21% from 1014 infected seeds and 6.7% from 2000 tested seeds. Similarly, 96 seeds were infected by *Curvularia lunata* that was 9.47% of total infected seeds and 4.8% of total tested seeds. The 54 seeds were infected by *Aspergillus* sp. that was 5.33% of total seed borne infection and 2.7% of total tested seeds. In case of *Chaetomium* sp 34 seeds were infected and frequency of occurrence was 3.35% and total tested seed infection was 1.7%. In case of *Nigrospora oryzae* 38 seeds were found infected, frequency of occurrence was 3.35% and total tested seed infection 1.9%. *Trichoderma* sp and *Aspergillus niger* infected in same proportion . For them 4 seeds were infected for each and frequency of occurrence was .4% and total tested seed infection 0.2% for each. However, Bacterial ooze were recorded in 52 seeds of rice. *Fusarium* sp, Unidentified mycelia, *Aspergillus flavus*, *Curvularia lunata*, *Aspergillus* sp, Bacterial ooze

are considered as predominant seed borne pathogens those cause more than 5% of total seed borne infections.

Table 2. Health status of HYV of rice seed (Aus) by inspection of dry seed method

Variety	Total weight (g)	Pure seeds (g)	Inert matter (g)	Other seed (g)	Fungal structures	Physical abnormalities of seed
BRRI dhan48	100	99.79	0.21	0.00	Present	Absent
BRRI dhan82	100	99.76	0.24	0.00	Present	Absent
BRRI dhan83	100	99.76	0.24	0.00	Present	Absent
BRRI dhan85	100	99.93	0.07	0.00	Present	Absent
BRRI dhan98	100	99.80	0.20	0.00	Present	Absent

Table3. Prevalence of seed borne pathogen of HYV of rice (Aus) identified by blotter method

Variety	% Pathogen incidence										% Seed infection
	Fusarium sp	*Curvularia lunata*	*Aspergillus flavus*	*A.* sp	*A.* niger	*Nigrospora oryzae*	*Chaetomium* sp	*Trichoderma* sp	Unidentified mycelial growth	Bacterial ooze	
BRRI dhan48	20	8	0	0	0	0.5	0	0	22.5	4.5	49.25
BRRI dhan82	11.5	4	17	9.5	0	0	2	0	16	0.5	46.25
BRRI dhan83	28.5	10	1.5	0	0	0	1	1	17	1	45.75
BRRI dhan85	18.5	0.5	4.5	1	1	4	0	0	7	7	39.75
BRRI dhan98	8.5	1.5	10.5	3	0	5	5.5	0	0	0	28.8

Table 4. Frequency and occurrence of various pathogens recorded from different varieties of HYV of rice seeds (Aus)by blotter method

Pathogen	No of pathogenic Infection[1]	% of total pathogen[2]	% of the seed yielding[3]	No of variety infected[4]
Fusarium sp	348	34.32	17.4	05
Aspergillus flavus	134	13.21	6.7	05
Curvularia lunata	96	9.47	4.8	05
Aspergillus sp	54	5.33	2.7	05
Bacterial ooze	52	5.13	2.6	05
Nigrospora oryzae	38	3.75	1.9	05
Chaetomium sp	34	3.35	1.7	05
Trichoderma sp	4	0.4	0.2	05
Asperillus niger	4	0.4	0.2	05
Mycelial growth	250	24.66	12.5	05

[1] Total no of seed borne pathogenic infections recorded from the seeds of five HYV of rice (Aus). It means, 348 seeds were infected by *Fusarium* sp from 2000 tested seeds of five HYV of rice seeds. Total number of seed borne pathogenic infection was 1014.

[2] % of total pathogenic infection was calculated from all seed borne infections of a crop. It is the percentage of total number of infected seeds (1014). It means, 34.32% seeds of total infected 1014 seeds were infected by *Fusarium* sp.

[3] Percentage of seed yielding of different pathogens was calculated on the basis of 2000 tested seeds. It is the percentage of total number tested seeds (2000). It means, 17.4% seeds of total tested 2000 seeds were infected by *Fusarium* sp.

[4] Total number of seed samples/tested variety was 05.

A

B

C

D

E

F

Figure 5. Dry inspection of BRRI dhan48; A. Seed sample; B. Insect damaged seeds; C. Fungal structure on seeds; D. Insert matter: E. Insects associated with seeds: F. Discolored seeds

A

B

C

D

E

F

Figure 6: Dry inspection of BRRI dhan82; A. Seed sample; B. Insect damaged seeds; C. Fungal structure on seeds; D. Inert matter; E. Insects associated with seeds; F. Discolored seeds

A

B

C

D

E

F

Figure 7. Dry inspection of BRRI dhan83; A. Seed sample; B. Insect damaged seeds; C. Fruiting structures on seeds; D. Inert matter; E. Insects associated with seeds; F. Discolored seeds

A

B

C

D

E

Figure 8. Dry inspection of BRRI dhan85; A. Seed sample; B. Fruiting structures on seeds; C. Inert matter; D. Discolored seeds; E. Insects associated with seeds

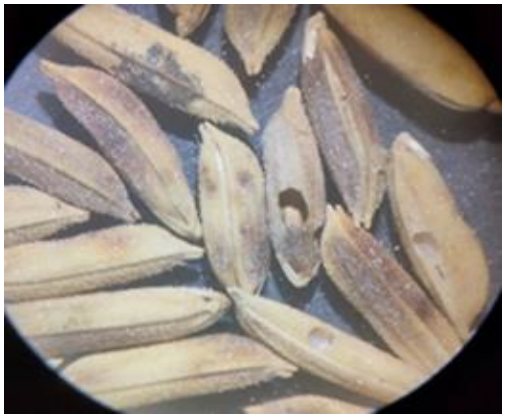
A

B

C

D

E



F

Figure 9. Dry inspection of BRRI dhan98; A. Seed sample; B. Insect damaged seeds; C. Fruiting structures (black) on seeds; D. Fruiting structures(white) on seeds; E. Inert matter; F. Discolored seeds

4.2 Seed Health Study of HYV rice (Aman) varieties

4.2.1 Health status of HYV rice (Aman) varieties by inspection of dry seed method

A. Physical appearance of seeds

No physical abnormalities were observed in Aman rice varieties (BRRI dhan49, BRRI dhan75, BRRI dhan87) (Table 5)

B. Presence of fruiting structures of fungi

Mycelial structures were found on the seed surface of selected Aman rice variety seeds. Some are visible even with a naked eye. (Table 5).

C. Purity analysis

For purity analysis, 100gram seeds of each variety were analyzed. The seeds were observed by naked eyes. Inert matters were found from different varieties, but no other seeds were found (Table 5).

4.2.2 Prevalence of seed-borne pathogen of HYV rice (Aman) varieties identified by blotter method

The pathogenic incidence varied significantly among the samples for all pathogens (Table 6)

A. Identified pathogen

Different fungal pathogens viz. *Aspergillus flavus*, *Fusarium* sp, *Curvularia lunata*, Unidentified mycelial growth *Pyricularia oryzae*, *Nigrospora oryzae*, *Rhizopus stolonifer*, Bacterial ooze were observed and recorded. Total 8 pathogens were identified from the seeds of three varieties of HYV varieties of Aman rice. (Table 5)

B. Incidence of seed borne pathogens

In table, in case of fungal infection, incidence of *Fusarium* sp was varied from 6.00% to 21.5%. Incidence of unidentified mycelial growth of unidentified pathogen was varied from 3.5% to 26.5%. Incidence of *Curvularia lunata* was varied from 2.5% to 17%. Incidence of *Aspergillus flavus* and *Rhizopus stolonifer* were varied from 0.5% to 10.5% and 1% to 17%. Incidence of *Pyricularia oryzae* was varied from 4% to 12%. Incidence of Bacterial ooze was varied from 4% to 8.5%. Incidence *Nigrospora oryzae* was 2% in BRRI dhan49. (Table 6).

C. Frequency and occurrence of various pathogen recorded in different variety of HYV of rice (Aman) seeds

In table 7, frequency of various pathogen occurrences was calculated in seed samples of different varieties of rice seeds (Aman). It was observed that, the highest 224 seeds from 1200 tested seeds of three varieties of HYV of rice (Aman) were infected by mycelial growth of unidentified pathogen, that was 29.5% of total seed borne infection (760) and 18.67% of the seed yielding

(1200). Moreover, 148 seeds were infected by *Fusarium* sp that was 19.47% of total seed infection and 12.33% of total seed yield. In case of *Pyricularia oryzae* 96 seeds were infected and the frequency of occurrence was 12.63% from 760 infected seeds and 8% from 1200 tested seeds. Similarly, 88 seeds were infected by *Curvularia lunata* that was 11.58% of total infected seeds and 7.33% of total tested seeds. The 70 seeds were infected by Bacterial ooze that was 9.21% of total seed borne infection and 5.83% of total tested seeds. In case of *Rhizopus stolonifer* 68 seeds were infected and frequency of occurrence was 8.95% and total tested seed infection was 5.67%. In case of *Aspergillus flavus* 58 seeds were found infected, frequency of occurrence was 7.63% and total tested seed infection 4.83% In case of *Nigrospora oryzae* 8 seeds were found infected, frequency of occurrence was 1.05% and total tested seed infection 0.67%.

Table 5. Health status of HYV of rice seed (Aman) by inspection of dry seed method

Variety	Total weight (g)	Pure seeds (g)	Inert Matter (g)	Other seeds (g)	Fungal Structure	Physical abnormalities of seed
BRRI dhan49	100	99.98	0.02	0.00	Present	Absent
BRRI dhan75	100	99.85	0.15	0.00	Present	Absent
BRRI dhan87	100	99.95	0.05	0.00	Present	Absent

Table 6. Prevalence of seed borne pathogen of HYV of rice (Aman) identified by blotter method

Variety	% Pathogen incidence								% Seed Infection
	Fusarium sp	*Curvularia lunata*	*Pyricularia oryzae*	*Nigrospora oryzae*	*Aspergillus flavus*	*Rhizopus stolonifer*	Unidentified mycelial growth	Bacterial ooze	
BRRI dhan49	6	17	8	2	0.5	0	26.5	4	50.25
BRRI dhan75	21.5	2.5	12	0.0	10.5	17	3.5	5	58.75
BRRI dhan87	9.5	2.5	4	0.0	3.5	1	26	8.5	50.25

Table 7. Frequency and occurrence of various pathogens recorded from different varieties of HYV of rice seeds (Aman)by blotter method

Pathogens	No. of pathogenic infection[1]	% of total pathogen[2]	% of the seed yielding[3]	No of variety tested[4]
Fusarium sp	148	19.47	12.33	03
Pyricularia oryzae	96	12.63	8.00	03
Curvularia lunata	88	11.58	7.33	03
Bacterial ooze	70	9.21	5.83	03
Rhizopus stolonifer	68	8.95	5.67	03
Aspergillus flavus	58	7.63	4.83	03
Nigrospora oryzae	8	1.05	0.67	03
Mycelial growth	224	29.5	18.67	03

[1]Total no of seed borne pathogenic infections recorded from the seeds of three varieties of HYV of rice (Aman). It means, 148 seeds were infected by *Fusarium* sp from 1200 tested seeds of three varieties of rice. Total number of seed borne pathogenic infection was 760.

[2] % of total pathogenic infection was calculated from all seed borne infections of a crop. It is the percentage of total number of infected seeds (760). It means, 19.47% seeds of total infected 760 seeds were infected by *Fusarium* sp.

[3] Percentage of seed yielding of different pathogens was calculated on the basis of 1200 tested seeds. It is the percentage of total number tested seeds (1200). It means, 12.33% seeds of total tested 1200 seeds were infected by *Fusarium* sp.

[4]Total number of seed samples/tested variety was 03.

A

B

C

D

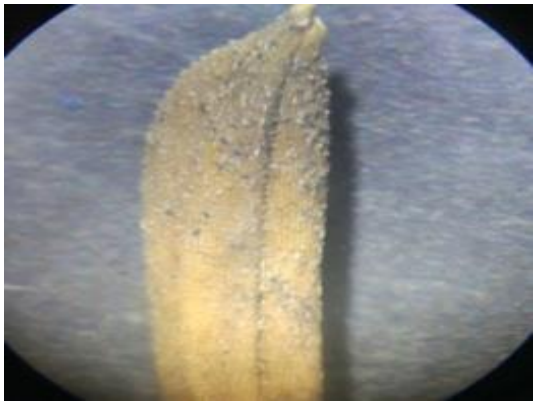
E

F

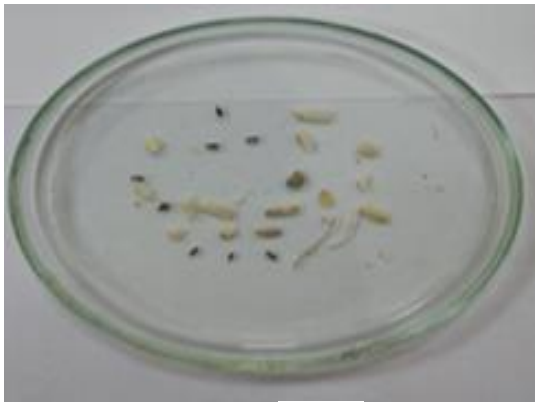
Figure 10. Dry inspection of BRRI dhan49; A. Seed sample; B. Insect damaged seeds; C. Fungal structures on seeds; D. Insert matter; E. Insects associated with seeds; F. Discolored seeds

A

B



C



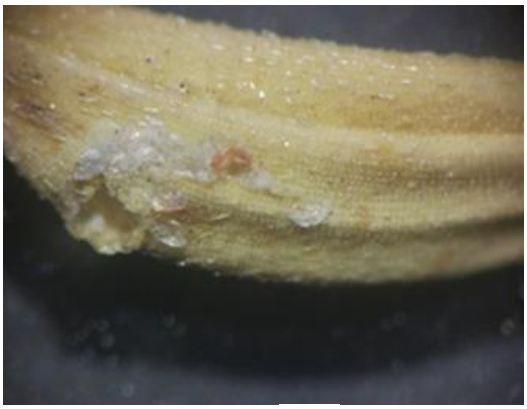
D

E

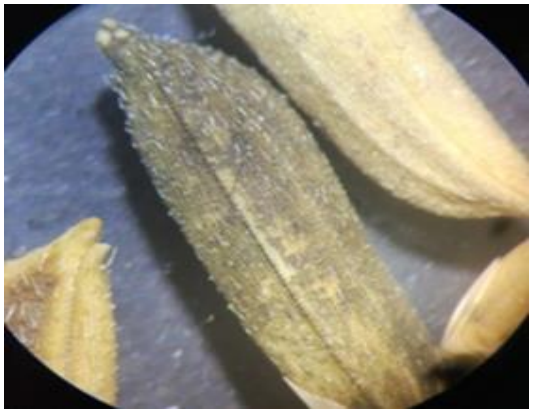
F

Figure 11. Dry inspection of BRRI dhan75; A. Seed sample; B. Insect damaged seeds; C. Fungal structures on seeds; D. Inert matter; E. Insects associated with seeds; F. Discolored seeds

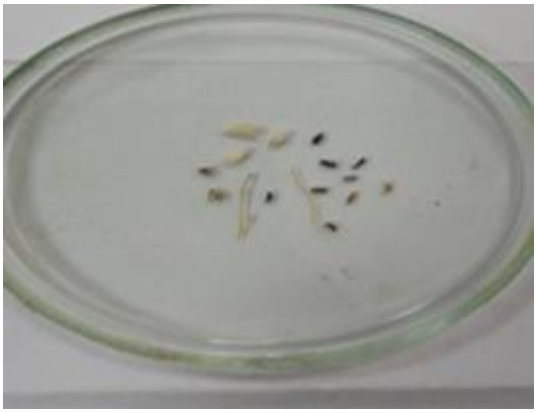
A



B



C



D

E

F

Figure 12. Dry inspection of BRRI dhan87; A. Seed sample; B. Insect damaged seeds; C. Fungal structures on seeds; D. Inert matter; E. Insects associated with seed; F. Discolored seeds

A. Physical appearance of seeds

No physical abnormalities were observed in Boro rice varieties BRRI dhan28 (2021), BRRI dhan29 (2021), BRRI dhan89, BRRI dhan28 (2022), BRRI dhan29 (2022) by naked eyes (Table 8)

B. Presence of fruiting structures of fungi

Mycelial structures were found on the seed surface of selected Boro rice variety seeds. Some are visible even with a naked eye (Table 8).

C. Purity analysis

For purity analysis, 100gram seeds of each variety were analyzed. The seeds were observed by naked eyes. Inert matters were found from different varieties, but no other seeds were found (Table 8).

4.3.2 Prevalence of seed-borne pathogen of HYV rice (Boro) varieties identified by blotter method

The pathogenic incidence varied significantly among the samples for all pathogens. BRRI dhab28 (2021), BRRI dhan29 (2021), BRRI dhan89, BRRI dhan28 (2022), BRRI dhan29 (2022) (Table 8)

A. Identified pathogens

Different fungal pathogens viz. *Aspergillus flavus*, *Fusarium* sp, *Aspergillus* sp, *Bipolaris oryzae*, *Aspergillus niger*, *Rhizopus solani*, Mycelia growth, *Nigrospora oryzae*, Bacterial ooze were observed and recorded. Total 9 pathogens were identified from the seeds of two varieties of HYV varieties of Boro rice. (Table 9).

B. Incidence of seed borne pathogens

In table 9, in case of fungal infection, incidence of bacterial ooze was varied from 1% to 3.00%. Incidence of mycelial growth of unidentified pathogen was varied from 2.5% to 7%. Incidence of *Bipolaris oryzae* was varied from 0.5% to 5.5%. Incidence of *Aspergillus flavus* and *Rhizopus stolonifer* were varied from 1.5% to 14.5% and 1 to 2%. Incidence of *Aspergillus* sp was varied from 1% to 6%. Incidence of *Aspergillus niger* was 4.5% in BRRI dhan29. Incidence *Fusarium* sp was 0.5% in BRRI dhan29. Incidence of *Nigrospora oryzae* was 2.5% in BRRI dhan28 (Table 9).

C. Frequency and occurrence of various pathogen recorded in different variety of HYV of rice (boro) seeds

In table 10, frequency of various pathogen occurrences was calculated in seed samples of different varieties of HYV of (boro) rice varieties. It was observed that, the highest 95 seeds from 2000 tested seeds of three varieties of HYV of rice (boro) were infected by *Aspergillus flavus*, that was 21.94% of total seed borne infection (433) and 4.75% of the seed yielding (2000). Moreover, 84 seeds were infected by unidentified mycelial growth

that was 19.40% of total seed infection and 4.2% of total seed yield. In case of *Bipolaris oryzae* 64 seeds were infected and the frequency of occurrence was 14.80% from 2000 infected seeds and 3.2% from 2000 tested seeds. Similarly, 56 seeds were infected by *Aspergillus sp* that was 12.93% of total infected seeds and 2.8% of total tested seeds. The 40 seeds were infected by Bacterial ooze that was 9.24% of total seed borne infection and 2% of total tested seeds. In case of *Aspergillus niger* 34 seeds were infected and frequency of occurrence was 7.90% and total tested seed infection was 1.7%. In case of *Rhizopus solani* 30 seeds were found infected, frequency of occurrence was 6.9% and 1.5% total tested seed infection. In case of *Nigrospora* sp, 24 seeds were found infected by it and the percentage were 5.5 and 1.2% of total infected seed and total tested seed respectively. Lastly 6 seeds were found infected by *Fusarium* sp and the percentage were 1.38 and 0.3% respectively.

Table 8. Health status of HYV of rice seed (Boro) by inspection of dry seed method

Variety (Year of collection)	Total weight (g)	Pure seeds (g)	Inert matter (g)	Other seeds (g)	Fungal structures	Physical abnormalities of seed
BRRIdhan28(2021)	100	99.63	0.37	0.00	Present	Absent
BRRI dhan29(2021)	100	99.95	0.05	0.00	Present	Absent
BRRI dhan28(2022)	100	99.92	0.08	0.00	Present	Absent
BRRI dhan29(2022)	100	99.82	0.18	0.00	Present	Absent
BRRI dhan89(2021)	100	99.93	0.07	0.00	Present	Absent

Table 9. Prevalence of seed borne pathogen of HYV of rice (boro) identified by blotter method

Variety	% Pathogen incidence									% Seed Infestation
	Bipolaris oryzae	*Aspergillus flavus*	*A.* sp	*A. niger*	*Rhizopus stolonifer*	*Fusarium* sp	*Nigrospora oryzae*	*Unidentified mycelial growth*	*Bacterial ooze*	
BRRI dhan28 (2021)	5	1.5	1	0	1	0	2.5	7	1	18.25
BRRI dhan29 (2021)	0.5	14.5	6	4.5	1	0.5	0	5	2.5	35.5
BRRI dhan89	4	5	2.5	0	2	0	1	3	1.5	17.75
BRRI dhan28 (2022)	5.5	2	1.5	0	2	0	2.5	3.5	2	22.25
BRRI dhan29 (2022)	1	8	3	4	1.5	1	0	2.5	3	16.25

Table 10. Frequency and occurrence of various pathogens recorded from different varieties of HYV of rice seeds (Boro)by blotter method

Pathogens	No. of pathogenic infection[1]	% of total pathogen[2]	% of the seed yielding[3]	No of variety sample[4]
Aspergillus flavus	95	21.94	4.75	05
Bipolaris oryzae	64	14.80	3.2	05
Aspergillus sp	56	12.93	2.8	05
Bacterial ooze`	40	9.24	2	05
Aspergillus niger	34	7.90	1.7	05
Rhizopus sp	30	6.9	1.5	05
Nigrospora oryzae	24	5.5	1.2	05
Fusarium sp	6	1.38	0.3	05
Mycelial growth	84	19.40	4.2	05

[1]Total no of seed borne pathogenic infections recorded from the seeds of five sample of three varieties of HYV of rice (Boro). It means, 95 seeds were infected by *Aspergillus flavus* from 2000 tested seeds of rice. Total number of seed borne pathogenic infection was 433.

[2] % of total pathogenic infection was calculated from all seed borne infections of a crop. It is the percentage of total number of infected seeds (433). It means, 21.94% seeds of total infected 433 seeds were infected by *Aspergillus flavus*.

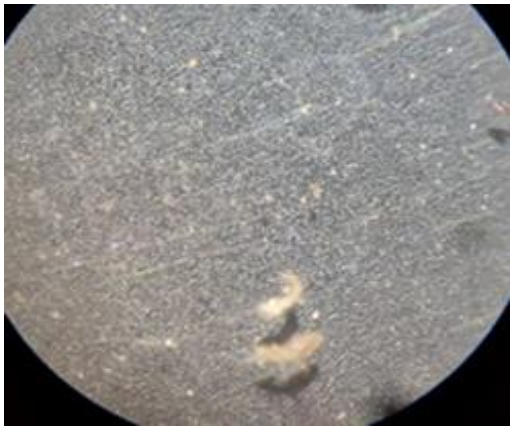
[3] Percentage of seed yielding of different pathogens was calculated on the basis of 2000 tested seeds. It is the percentage of total number tested seeds (2000). It means, 4.75% seeds from total tested 2000 seeds were infected by *Aspergillus flavus*.

[4]Total number of seed samples was 05.

A

B

C



D

E

Figure 13. Dry inspection of BRRI dhan28 (2021) (Boro); A. Seed sample; B. Insect infected seeds; C. Discolored seeds; D. Inert matter; E. Fruiting structures on seed

A

B

C

D

E

Figure 14. Dry inspection of BRRI dhan29 (2021); A. Seed sample; B. Insect infected seeds; C. Discolored seeds; D. Inert matter; E. Stereo microscopic view of inert matter

A

B

C

D

Figure 15. Dry inspection of BRRI dhan28(2022); A. Seed sample; B. Insect infected seeds; C. Fungal structure on seeds; D. Discolored seeds

A

B

C

D

E

Figure 16. Dry inspection of BRRI dhan29 (2022). A. Seed sample; B. Fungal structure on seeds; C. Insect infected seeds; D. Inert matter; E. Discolored seeds

A

B

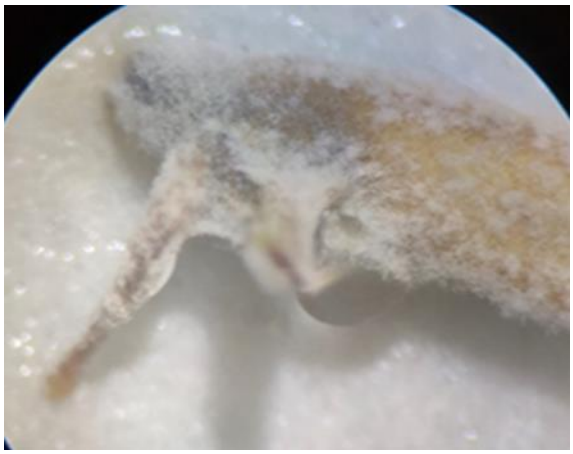
C

D

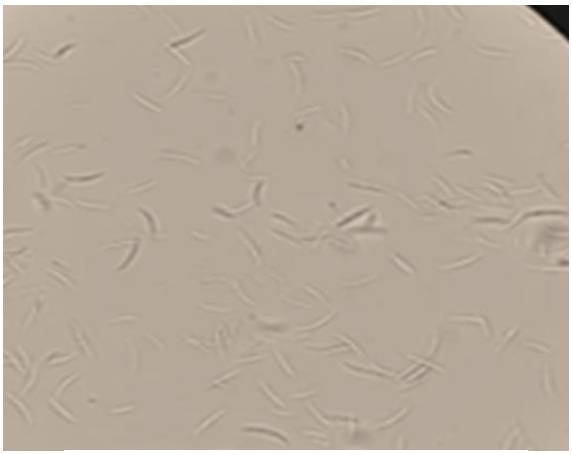
E

F

Figure 17. Dry inspection of BRRI dhan89; A. Seed sample; B. Insect infected seeds; C. Fungal structures on seeds; D. Insects of seeds; E. Inert matter; F. Discolored seeds

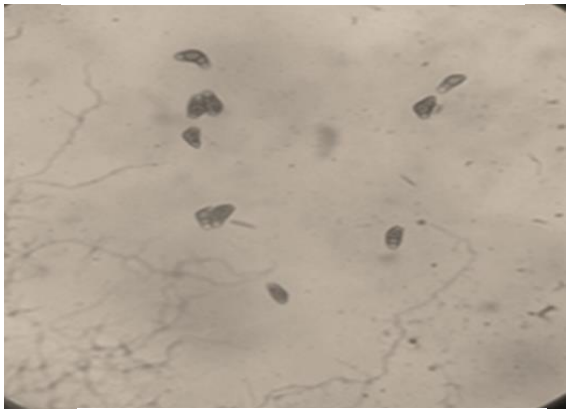


A. Growth of *Fusarium* sp on seed

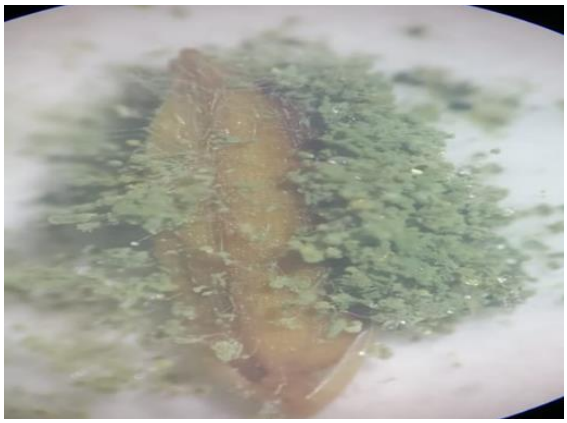


B. Spore of *Fusarium* sp

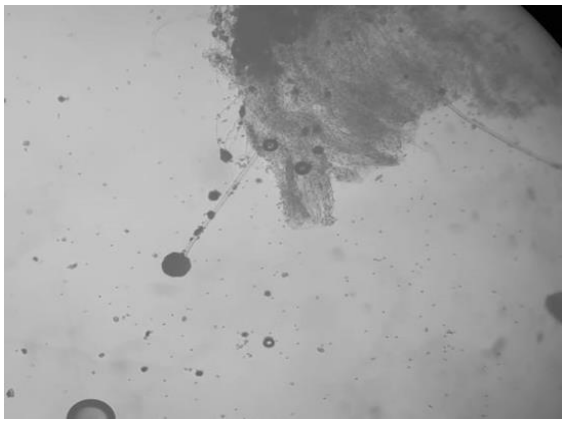
C. Growth of *Curvularia lunata* on seed



D. Spore of *Curvularia lunata*

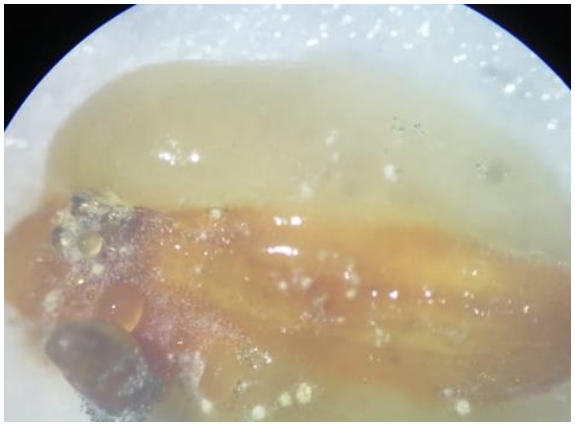


E. Growth of *Aspergillus flavus* on seed



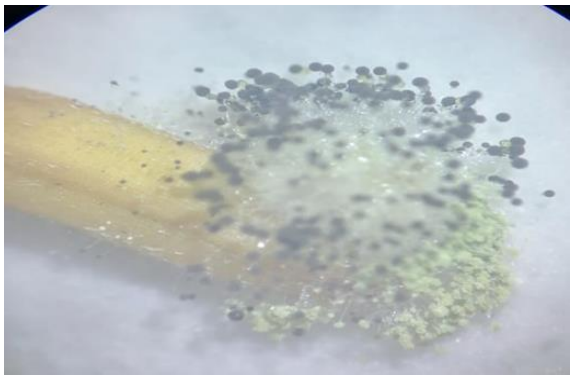
F. Spore of *Aspergillus flavus*

Figure 18. Stereo and compound microscopic view of identified pathogens of rice seeds (Aus, Aman , Boro); A. Growth of *Fusarium* sp B. Spore of *Fusarium* sp ; C. Growth of *Curvularia lunata* D. Spore of *Curvularia lunata* ; E. Growth *Aspergillus flavus* ; F. Spore of *Aspergillus flavus*



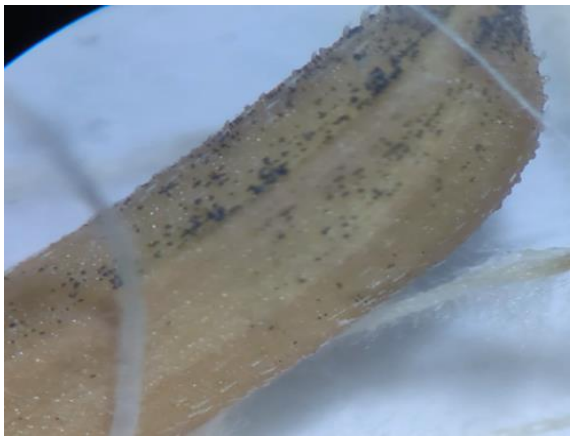
G. Bacterial ooze

H. Growth of *Chaetomium* sp on seed

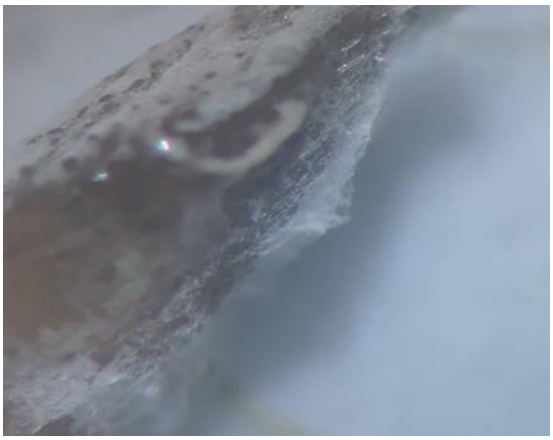


I. Growth of *Aspergillus niger on seed*

J. Growth of *Aspergillus* sp on seed

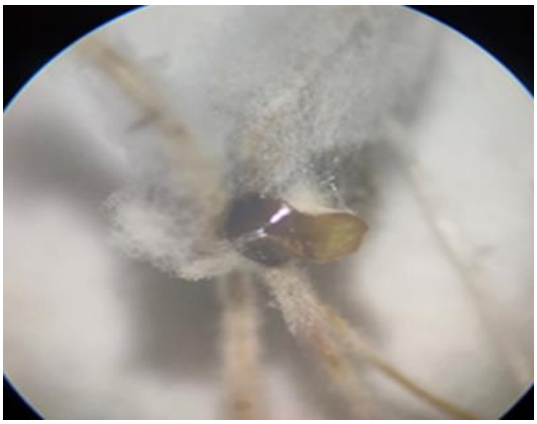


K. Growth of *Nigrospora oryzae* on seed

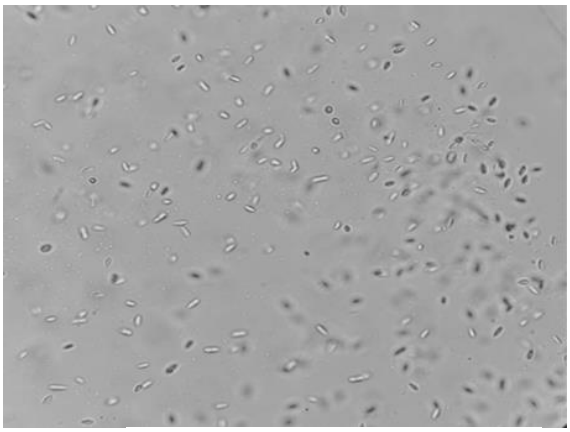


L. Unidentified fungal mycelial growth

Figure 19. Stereoscopic view of identified pathogens of rice seeds (Aus, Aman , Boro); G. Growth of Bacterial ooze ; H. Growth of *Chaetomium* sp ; I. Growth of *Aspergillus niger* J. Growth of *Aspergillus sp*; K. Growth of *Nigrospora oryzae* ; L. Unidentified fungal mycelial growth

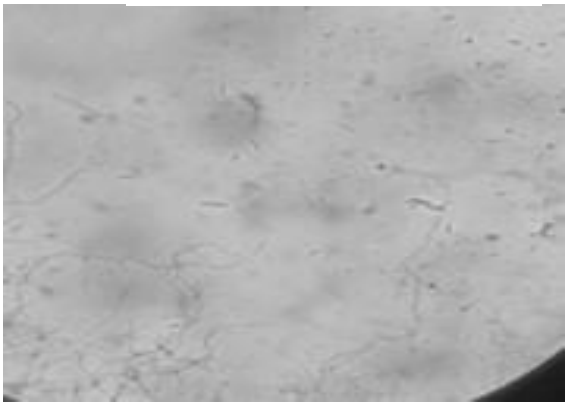


A. Growth of *Fusarium* sp on seed

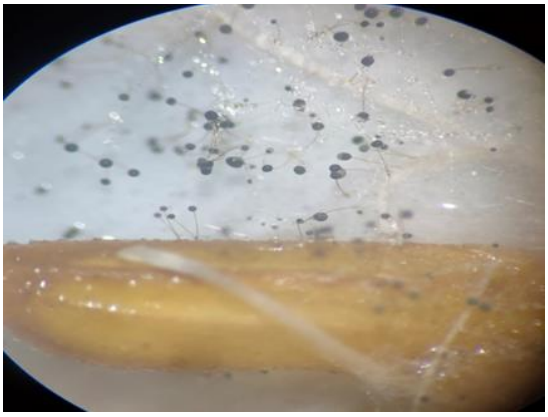


B. Spore of *Fusarium* sp

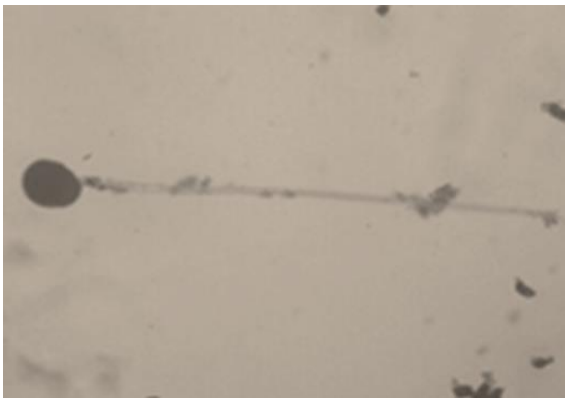
C. Growth of *Pyricularia oryzae on seed*



D. Spore of *Pyricularia oryzae*

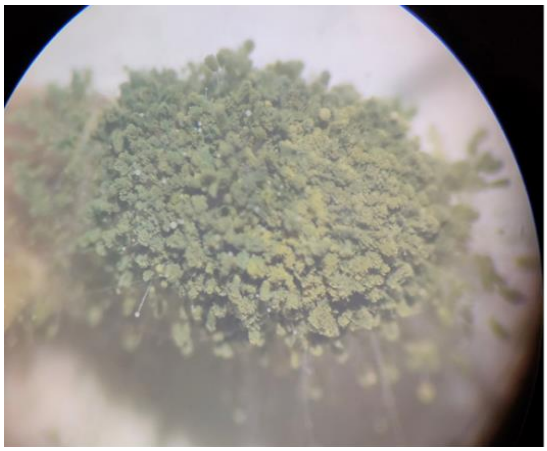


E. Growth of *Rhizopus stolonifer on seed*

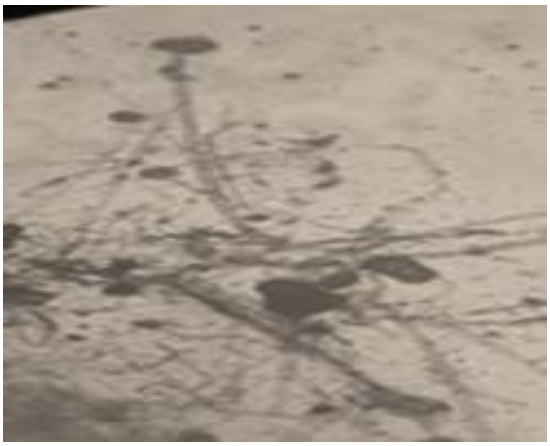


F. Structure of *Rhizopus stolonifer*

Figure 20. Stereo and compound microscopic view of predominant identified pathogens of rice seeds (Aman); A. Growth of *Fusarium* sp B. Spore of *Fusarium* sp ; C. Growth of *Pyricularia oryzae* D. Spore of *Pyricularia oryzae* ; E. Growth *Rhizopus stolonifer* ; F. Structure of *Rhizopus stolonifer*



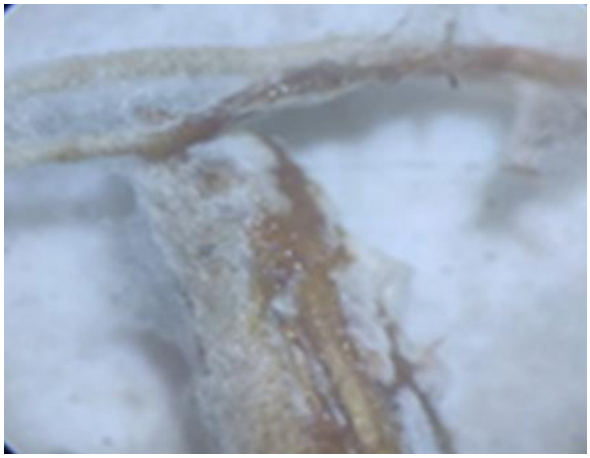
A. Growth of *Aspergillus flavus* on seed



B. Spore of *Aspergillus flavus*

C. Growth of *Bipolaris oryzae on seed*

D. Spore of *Bipolaris oryzae*



E. Unidentified fungal mycelial growth

Figure 21. Stereo and compound microscopic view of predominant identified pathogens of rice seeds (Boro); A. Growth of *Aspergillus flavus* ; B. Spore of *Aspergillus flavus* ; C. Growth of *Bipolaris oryzae ;*D. Spore of *Bipolaris oryzae*; E. Unidentified fungal mycelial growth

DISCUSSION

Eleven varieties of HYV of rice seed viz BRRI dhan48, BRRI dhan82, BRRI dhan83, BRRI dhan85, BRRI dhan98, BRRI dhan49, BRRI dhan75, BRRI dhan87, BRRI dhan28, BRRI dhan29, BRRI dhan89 were examined to determine the health status of seeds collected from Bangladesh Agricultural Development Corporation (BADC), Dhaka, Bangladesh.

For seed health study, recommended procedure of 'International Rules for Seed Testing (ISTA, 1999) was followed. In inspection of dry seed method, pathogenic structures, fruiting body and physical abnormalities viz. deformed, shrinkage, swelling, spotted and undersized seeds were recorded. In blotter plate method, 400 seeds of each variety were thoroughly investigated and the incidence and frequency of identified pathogen has been recorded.

A considerable amount of seed borne fungi and unidentified pathogens were observed by inspection of dry seed and blotter method of seed health testing. Altogether nine genera of seed borne fungal pathogens viz. *Pyricularia*, *Aspergillus*, *Curvularia*, *Fusarium*, *Nigrospora*, *Trichoderma*, *Chaetomium*, *Bipolaris* and *Rhizopus* were recorded eleven varieties HYV of rice.

In dry inspection method, insect infected and discolored seeds were observed in different varieties seeds. Powdery structure of fungus was observed on many seeds. For each variety, inert matters were found but no other seeds were found. In blotter method, prominent seed borne pathogens were identified, namely *Aspergillus flavus, Aspergillus niger*, *Aspergillus* sp., *Fusarium s*p.,

Curvularia spp., *Chaetomium* sp., *Bipolaris oryzae*, *Nigrospora* sp., *Trichoderma* sp and *Rhizopus* sp. in total. A number of unidentified pathogens and bacterial ooze were also observed.

In dry inspection of Aus rice, no other crop seeds were found in any variety. We tested five HYV of rice seed (Aus) and found that pure seed (99.76 to 99.93%), inert matter (0.07 to 0.24%). Akter and Hossain (2015) conducted an experiment and reported that, pure seed ranged from 92.86 to 100%, other seeds 0 to 7.14% and inert matter 0 to 2.59%. Through blotter method 10 pathogens were observed. The pathogens were *Aspergillus flavus*, *Aspergillus niger*, *Fusarium moniliforme*, *Curvularia lunata*., *Aspergillus* sp, *Nigrospora oryzae*., *Chaetomium* sp, *Trichoderma* sp, Bacterial ooze and unidentified mycelial growth. The highest total pathogen infection (49.25%) was in BRRI dhan48, whereas lowest (28.8%) was in BRRI dhan98. The frequency and occurrence of various pathogens in different variety of HYV rice seed (Aus) were also recorded through blotter plate method. The highest frequency of fungi was *Fusarium moniliforme* (34.32%) whereas lowest frequency was *Aspergillus niger* (.4%). Islam *et al*. (2012) also isolated *Fusarium moniliforme*, *Trichoconis padwickii*, *Aspergillus flavus*, *Curvularia lunata*, *Bipolaris oryzae*, *Rhizopus* sp., *Aspergillus niger*, *Aspergillus clavatus*, and *Chaetomium* sp. Mansur *et al.* (2013) conducted an experiment and found *Fusarium oxysporum*, *F. moniliforme*, *Bipolaris oryzae*, *Alternaria padwickii*, *Curvularia lunata*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp. and *Nigrospora oryzae*.

In dry inspection of Aman rice seeds, seeds from other crops were absent. We tested three varieties of rice seed (Aman) for seed health testing . The range of pure seed was (99.85-99.98%) and inert matter was (0.02-0.05%). Pain *et al*. (2022) conducted an experiment where they worked on some transplanted Aman rice varieties. The range of healthy seed was (0.36g-5.58g), inert matter (0.00g- 0.24g). In blotter method, eight different pathogens were found. They were *Fusarium oxysporum*., *Curvularia lunata*., *Pyricularia oryzae*, *Nigrospora* sp, *Aspergillus flavus*, *Rhizopus stolonifer*, Bacterial ooze and unidentified mycelial growth. The highest total seed borne infection (58.75%) were recorded in BRRI dhan75. However, in BRRI dhan49, the total infection was 50.25%. BRRI dhan87 also showed the same. The present study also revealed that, the highest infection occurred due to *Fusarium oxysporum*. (19.47%) among all identified fungi. On the other hand, the second highest infection occurred due to unidentified mycelial growth that was (29.5%). However, the frequency of *Pyricularia oryzae*, *Curvularia lunata*, Bacterial ooze, *Rhizopus stolonifer*, *Aspergillus flavus*, *Nigrospora oryzae* were 12.63%, 11.58%, 9.21%, 8.95%, 7.63%, 1.05% respectively. Mansur *et al.* (2013) also conducted an experiment on seed health status of rice seeds and identified nine different pathogens viz. *Fusarium oxysporum*, *Fusarium moniliforme*, *Bipolaris oryzae*, *Alternaria padwickii*, *Curvularia lunata*, *Aspergillus flavus*, *Aspergillus niger*, *Penicillium* spp. and *Nigrospora oryzae*. Hossain *et al*. (2020) also identified *Fusarium moniliforme* as one of the major seed borne pathogens in Bangladesh.

In dry inspection of Boro rice varieties, the pure seeds ranged from 99.63 to 99.95% and inert matter ranged from 0.05 to 0.37%. No other crop seeds were found. Cottony like fungal structures were present. Haque *et al.* (2007) conducted an experiment and performed different kinds of seed health testing including dry inspection and blotter method and found 99.01% of pure seed in seed samples of trained farmer and minimum (96.19%) in untrained farmers. The present study found 8 different fungi that were associated with Boro rice varities through blotter plate method. The highest seed borne infection found in BRRI dhan29 and lowest seed borne infection occurred in BRRI dhan89. However, in case of total pathogenic infection *Aspergillus flavus* (21.94%) was found to be the most prominent in each variety and lowest infection occurred due to *Fusarium moniliforme* that was 1.38%. Gopal Krishna (2010) reported about 8 genera of fungi. Among them the most predominant one was *Bipolaris oryzae* (58.89%) followed by *Alternaria padwickii* (52.96%). Bhuiyan *et al* (2013) conducted an experiment on BRRI dhan28 and BR11. They identified 6 different fungi which were *Curvularia lunata*, *Sarocladium oryzae*, *Alternaria padwickii*, *Bipolaris oryzae*, *Aspergillus niger*, and *Fusarium* spp. The present study also revealed that altogether 8 different fungi viz. *Aspergillus flavus*, *Bipolaris oryzae*, *Aspergillus* sp., Bacterial ooze, *Aspergillus niger*, *Rhizopus* sp, *Nigrospora oryzae*., *Fusarium moniliforme* were found to be associated with boro rice varieties.

CHAPTER 5

SUMMARY AND CONCLUSION

Rice is a major source of sustenance in terms of providing food, income and employment in Bangladesh. It covers about most of the total cropped area in the country and it is the most important cereal crop as well as staple food for about 160 million people of Bangladesh.

The population of the country is increasing but climate change issues have become the major hindrance which is responsible low production of agricultural units globally. To ensure food security, high yielding variety of rice has become the source of sustainability. But past few years due to seed borne diseases, rice production has been facing challenges and thus millions of farmers are being affected every year. Considering the important of seed borne pathogen, this study was conducted to detect and identify the seed borne pathogens of some selected high yielding variety of rice supplied by BADC farm. The investigation was carried out in the Seed Health Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period of July 2021 to June 2022. This study was carried out according to the rules of International Seed Testing Agency (ISTA) with selected HYV of rice namely BRRI dhan48, BRRI dhan82, BRRI dhan83, BRRI dhan83, BRRI dhan85, BRRI dhan98, BRRI dhan49, BRRI dhan75, BRRI dhan87, BRRI dhan28, BRRI dhan29, BRRI dhan89 from BADC farm.

Two common seed health testing methods viz. inspection of dry seed method and blotter method were followed according to the rules of ISTA. Through inspection of dry seed method, farmers normally get primary idea about seed value of loose seeds by observing visually. On the other hand most of the seed borne pathogens can easily be detected and identified through blotter plate method. Thus, this method is the most acceptable method of seed health testing used through worldwide. Inspection of dry seed method showed that highest

purity percentage found in BRRI dhan49 (99.98%) whereas highest inert matter percentage (0.37%) found in BRRI dhan28. However, physical abnormalities of seed were absent but fungal structures, discolored seed, insect damaged seed, insects were present in seed.

In blotter method, out of the 13 tested seed samples of eleven varieties 11 pathogens were detected. The recorded fungi were *Bipolaris oryzae*, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus* sp., *Curvularia lunata*, *Fusarium oxysporum*, Bacterial ooze, *Nigrospora* sp., *Chaetomium* sp., *Pyricularia oryzae*, *Rhizopus* sp and unidentified pathogens. Higher seed infection found in BRRI dhan48, BRRI dhan75, BRRI dhan29 among Aus, Aman, Boro varieties respectively. The present research finding indicated that, infestation of seed borne fungi were varied from variety to variety.

The incidence of seed borne pathogen also varied from variety to variety. By determining the frequency and occurrence of various pathogens.it can be said that most of the seed samples mainly infected *by Fusarium oxysporum* and *Aspergillus flavus*. Unidentified mycelial growth, Bacterial ooze, *Bipolaris oryzae*, *Curvularia lunata* also found in great extent.

High yielding varieties (HYV) of rice has become the major source of rice seed in achieving food security in Bangladesh. Bangladesh Agricultural Development Corporation (BADC) supply major portion of rice seed against seed demand. The present study showed that a number of seed pathogens were associated with different variety of HYV rice seed. Several reasons are responsible for seed borne pathogenic infection. Among them, poor management of storage condition responsible in a great extent.

Taking overall findings into considerations, it can be said that health status of HYV rice seeds from BADC needs to be more pathogen free. That’s why, further study will need to be carried out.

CHAPTER 6

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