DETECTION AND IDENTIFICATION OF SEED BORNE PATHOGENS OF IMPORTED HYBRID RICE VARIETIES IN BANGLADESH

BY

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CERTIFICATE

This is to certify that the thesis entitled "**DETECTION AND IDENTIFICATION OF SEED BORNE PATHOGENS OF IMPORTED HYBRID RICE VARIETIES IN BANGLADESH**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE IN PLANT PATHOLOGY**, embodies the results of a piece of bonafide research work carried out by **NURA ORA, REGISTRATION NO. 04-01365**, under my supervision and guidance. No part of this thesis has been submitted for any other degree in any other institutions.

I further certify that any help or sources of information received during the course of this investigation have been duly acknowledged.

Dated: December, 2009
Dhaka,Bangladesh

Mrs. N. Akhtar

Mrs. Nasim Akhtar
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Dedicated to my
Beloved Parents
&
Elder sister

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ABSTRACT

The experiment was carried out at Sher-e-Bangla Agricultural University during 2008-09 according to the ISTA rules following blotter method, agar plate method and rolled paper towel method. Thirteen imported hybrids, two local hybrids and two local high yielding varieties were tested to determine the seed health status. A total of twelve pathogens were associated with the collected seeds viz. *Xanthomonas* spp, *Rhizopus stolonifer, Aspergillus* spp., *Bipolaris oryzae, Fusarium moniliforme, Curvularia lunata, Penicillum* sp., *Tilletia barclayana, Alternaria tenuissima, Chaetomium globosum, Phoma* sp and *Nigrospora* sp. The incidence of different pathogens vary individually and independently among the hybrids and local varieties of rice seeds. Seed germination varied from 54.63%- 99.50% in blotter method. The incidence range of *Xanthomonas* spp. (0 -18.13%), *Rhizopus stolonifer* (0-19.75%), *Aspergillus* spp. (0-12.00%), *Bipolaris oryzae* (0-18.00%), *Curvularia lunata* (0-4.25%), *Penicillum* sp. (0-1.75%), *Tilletia barclayana* (0-2.75%) , *Alternaria tenuissima* (0-0.63%), *Chaetomium globosum* (0-1.25%), *Phoma* sp (0-0.50%) and *Nigrospora* sp.(0-0.25%) were detected by blotter method. Seed germination ranged from 8.25%-96.38% was recorded in rolled paper towel method. Non germinated hard seed ranged from 1.63%-86.50% and rotten seed 0.75%-12.00%. Post emergence mortality was observed 0-4.38% by rolled paper towel method. The vigour index ranged from 96.36%-2329.28%. The incidence range of *Xanthomonas* spp. (1.63%-5.13%), *Rhizopus stolonifer* (0-3.00%), *Bipolaris oryzae* (0-15.13%), *Fusarium moniliforme* (0-7.63%), *Curvularia lunata* (0-6.38%), *Penicillum* sp.(0-1.63%), *Alternaria tenuissima* (0-1.63%), *Aspergillus flavus* (0-6.50), *Aspergillus niger* (0-1.38%) sp and *Nigrospora* sp.(0-1.38%) was detected by agar plate method. The detected predominant pathogens were *Xanthomonas* spp., *Bipolaris oryzae, Aspergillus* sp. *Fusarium moniliforme ,Rhizopus stolonifer.*

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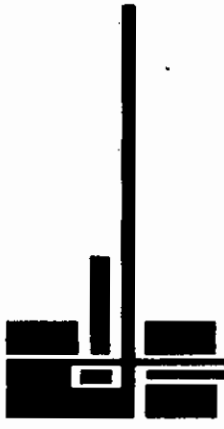


Chapter 1
Introduction

CHAPTER 1

INTRODUCTION

Rice is one of the world's primary food crops mostly grown in tropical and sub tropical climate. In Asia, rice is the principal staple food and the most important source of employment and income for rural people. Asia's hot and humid climate during the long and heavy monsoon season provide the most favorable agro ecological environment for rice cultivation, as well as disease development.

Rice is the staple food for about 144.5 million people of Bangladesh. Rice is cultivated in 10.58 million hectare of land covering 80% arable land and accounts for 95% food grain production in Bangladesh (BBS, 2008). Although rice is the staple food for people of Bangladesh, its yield is relatively low compared to those of other rice growing countries. In Bangladesh, 28.93 million tons of rice is produced with average yield around 2.74 t/ha; whereas world average yield 4.15 t/ha and average yield 4.21 t/ha in Asia (BBS, 2008 and FAO, 2008). This result indicates that average per hectare production of rice in Bangladesh is extremely low compared to other rice growing countries of the world. So there is no alternative to increase the yield of rice to fulfill the future national demand. One of the best options available to the plant breeder is the hybrid rice.

The Bangladesh Government has heavily promoted hybrid rice cultivation, and aims to boost hybrid rice production from 250,000 hectares in last planting season to One million hectares in 2008. First it was considered as an alternative technology for breaking the present yield

ceiling of modern varieties. But the yields of these hybrid varieties are hampered by different factors. In Bangladesh most of the hybrid rice seeds are imported from China. Major constrains in hybrid rice adoption were identified; these were high cost of seed, requirement of more crop care and management time, less grain yield, high pest and disease attack, low profits and lack of suitability for home consumption (AAS, 1999).

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 important characteristics of seed quality, purity, germination, high yielding potentiality and seed health quality are of major importance. Of these major characteristics of a quality seed, health is immensely important. Seed health refers to whether a seed or a seed lot is infected by pathogens or not. Infected seeds fail to germinate and the pathogen from the infected seeds may be transmitted to seedlings and growing plants in the field causing disease. Therefore, it is important to know whether a seed lot is free from seed-borne infection of pathogen (s) or the lot contains pathogen (s) with its maximum acceptable limit. This has great value to the growers. Because even a pure viable seed of high yielding variety is of little or no use to the growers, if the seed is unhealthy or infected by a virulent pathogen. In fact, under favorable conditions such infected seeds can create disease epidemic in the field resulting partial to total crop failure.

Pathogen free seed is the vital input in agriculture. The average yield 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 TK. 12 thousand millions is lost due to diseases caused by seed borne pathogens (Fakir *et al.* 2003). Without improving seed quality, 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 out by the seed and there is a great possibility to remain pathogen within the seed.

Seed is a 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 (Anslem 1981.). Seed-borne diseases create a great threat to the production of crops in Bangladesh. As many as 490 seed borne diseases are known to attack 756 different crop plants in Bangladesh of which at least 200 are of major concern (Fakir, 1991). Most of the major diseases of rice are seed borne (Fakir, 2002). 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. Fungi are the principal organisms associated with seed in storage. Of the seed borne diseases of rice, 22 are caused by fungi (Fakir., 2000). Bacteria are also commonly carried internally and externally by the seeds. The extremely seed borne pathogens of rice are Brown spot (*Bipolaris oryzae*), Bakanae (*Fusarium moniliforme*), Blast (*Pyricularia oryzae*), Sheath blight (*Rizoctonia solani*), Sheath rot (*Sarocladium oryzae*), Stem rot (*Sclerotium oryzae)* are associated with seed infection of rice and causes yield reduction, quality deterioration and germination failure (Mia *et al.*, 1979 and Shahjahan *et al.,* 1988).

Rice seed play an important role to carry pathogen in quarantine aspect. Farmers generally use different hybrid rice varieties and face the difficulties of many diseases. In the last few years the cultivation of

imported hybrid rice in Bangladesh increasesd rapidly. Recently Bacterial leaf blight (*Xanthomonas oryzae* pv. *oryzae,*), Bacterial leaf streak (*Xanthomonas oryzae* pv. *oryzicola*) disease appeared seriously in the boro rice. Objection was made from some corner that those diseases extremely appear in the hybrid rice variety. As the pathogens of BLB and BLS are seed borne, there is a chance to transmit new race of the pathogen in the country by imported hybrid rice seed. So assessment of the seed health standard of imported hybrid rice is very important for farmer and food security.

Considering the above facts the present experiment has been undertaken with hybrid rice varieties collected from the seed importer and local market of the country with the following objectives:

I) To identify different seed borne pathogens and their incidence from imported hybrid rice varieties in Bangladesh.

II) To know the comparative seed health status of hybrid rice varieties in Bangladesh.

Chapter 2
Review of Literature

CHAPTER 2

REVIEW OF LITERATURE

Rice, the main cereal crop suffers by different seed borne diseases. Pathogenic fungi and bacteria are the most responsible as well as limiting factors in cultivation of the crop in many part of the world. Many researches have been carried out in relation to seed health status and quality of different rice cultivars. However, some of the available literatures and relevant informations on seed health and quality have been cited in this chapter.

Malaya Cherewick (1954) found a number of fungi associated with rice seed. The fungi in order of prevalence were, *Helminthosporium oryzae, Nigrospora* spp., *Curvularia* spp., *Cercospora oryzae, Pyricularia oryzae, Fusarimn* spp., *Peslalotia oryzae, Coniothyrum* spp. and few other unidentified fungi. The incidence of these pathogens varied with year and place depending on the weather condition during maturing and harvesting.

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

Neergaard and Saad (1962) evaluated the practical applicability of blotter and agar plate methods for the development of routine seed health testing technique of rice seed in the laboratory. They compared three methods the standard blotter method, the 2, 4-D blotter method and the Agar planting method (PDA). They found that incubation at 22°C of 6 days was preferable for the blotter method and incubation at 28° C for 8 days

for the agar plate method for detecting the common seed-borne fungal pathogens of rice. The modified blotter method using 0.1% 2,4-D was found advantageous for inhibiting the sprouting of the seeds without hampering the growth of pathogens.

Augiero *et al.* (1966) reported that germination failure, foot rot, coleoptiles and stem rot and seedling blight of rice were attributed by a number of seed borne fungi. They observed that the fungi were *Fusarium moniliforme, Penicillium* spp., *Trichoconis padwickii* and *Helminthosporium oryzae* in order of prevalence.

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

Mathur *et al.* (1972) reported that about 73.5% of 388 tested rice seed samples from eleven countries were found to be infected with *Trichoconis padwickii.* Samples from Egypt, India, Korea, Nepal and Thailand showed heavy infection, upto 80% and infected seed had poor germination. The pathogen caused rotting in seeds, root and coleoptile with ultimate death of young seedlings. They also observed that infected seeds sown in pots resulted in considerable losses (rotting of seeds and seedling mortality) amounting to nearly half of the infection counts made on the laboratory.

Nanda and Chaudhary (1972) found 19 fungi associated with discoloured seed that reduced germinability and vigour of rice seeds. *Curvularia lunata (Cochliobolus lunatus)* was the most common (37%), followed by *Fusarium* spp. (13%) and *Chaetomium globosum* (6%).

According to Ou (1972), fungi associated with rice grains may be grouped into two, field fungi and storage fungi. He listed *Alternaria padwickii, Cephalosporium* spp., *Cladosporium* spp., *Epicoccum* spp., *Fusarium* spp., *Gibberella fujikuroi, Gibberella sosea, f cerealis, Helicoceras oryzae, Nigrospora* spp., *Phoma* spp., *Pyricularia oryzae* as field fungi and *Absidia, Aspergillus, Chaetomium, Mucor, Penicillium* and *Rhizopus* spp. as storage fungi. He also stated that in severely affected fields grain are infected by bacteria producing distinct symptom on the grains especially on the glumes.

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

Agarwal and Singh (1974) observed 7 fungal species with *Trichoconis padwickii* as the most common one. They also observed varietal differences on the incidence of seed borne fungi. Seeds of IR8 had the least infection and highest infection was recorded on Krishna. Grain discolouration was associated with heavy infection of *Curvularia lunata, Fusarium moniliforme, Fusarium semitectum, Trichoconis padwickii,* and *Trichothecium* sp.

Fakir and Ahmed (1974) investigated the association of seed borne mycoflora 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. *Aspergillus* spp. (26.7%), *C. lunata* (21.7%). *Tilletia barclayana* (19.1%), *Alternaria tenuis* (7.5%), *H. oryzae* (4.3%), *Fusarium* spp. (3.5%),

Chaetomium spp. (0.7%), *Zygorhynchus* sp. (0.7%), *Rhizopus nigricans* (0.6%), *Penicillium* spp. (0.5%) and *Sordaria* spp. (0.1%).

Hossain and Fakir (1974) studied on the seed microflora of freshly harvested rough rice of Aus varieties, which revealed the association of ten fungal genera. In order of prevalence these were *Curvularia, Aspergillus, Rhizopus, Fusarium, Alternaria, Nigrospora, Chaetomium, Sordaria. Helminthosporium* and *Penicillium. Curvularia,* the most predominant genus, constituted 28.9% of the total fungal isolation and 59.5% of the grains yielded this fungus. The total as well as the individual genera of fungi varied markedly and independently of each other with respect to variety, stage of collection medium and treatment of grains prior to isolation.

Esuruoso *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 *Drechslera oryzae, Pyricularia oryzae* and *Trichoconis padwickii* were seed-borne including some other fungi.

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

Shrestha *et al.* (1977) isolated *Alternaria, Cercospora, Curvularia, Drechslera, Epicoccum, Fusarium, Myrothecium, Nigrospora, Phaeotrichoconis, Pyricularia, Phoma* and *Trichoconis* from rice seed.

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

Reddy and Khare (1978) in India, noted four fungi in 42 rice seed samples collected from 41 districts, of which *Drechslera oryzae* and *Trichoconis padwickii* were 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.

Ashokan *et al.* (1979) studied on the influence of seed borne fungi on germination and post emergence mortality of rice (ADT 31) and Ragi (Co7) seedlings on treatment of seed with spore suspensions of 12 fungi *Helminthosporium* sp., *Curvularia* sp. and *Fusarium* sp, were most inhibitory on rice seed germination.

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.

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 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%).

Supriman and Palmar (1980) surveyed over 133 seed samples collected from Java, Bali and South Sulawesi, Indonesia, 21 fungal species comprising of 15 genera were isolated. Among the fungi most common were *Trichoconis padwickii, Curvularia lunata* and *Fusarium semitectum.* They also observed varietal differences and found lower susceptibility of IR26 and C4-63 to different fungi.

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.

Mia and Mathur (1983) investigated 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.

Sovae *et al.* (1983) reported the association of *Alternaria tenuis, Cladosporium herbarum, Curvularia lunata, Epicoccum purpurascens,*

Helminthosporium oryzae, Phoma spp., *Rhozoctonia solani* and *Pyricularia oryzae* in rice seed. The average incidence of these fungi were 12%, 13%, 3%, 28%, 2%, 6%, 33%, and 1%, respectively.

Kim *et al* (1984) found that the fungus *Monographella albescens* occurred at a frequency of 1-4% in 22 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 seed rot, seedling blight and brownish discoloration of the coleoptiles, primary and 2nd leaf when infected seeds were sown in agar or in soil.

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

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

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

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

Basak and Mridha (1988) studied the mycoflora associated with 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 isolated *Rhizopus* spp. had the maximum prevalence in seeds.

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

Gajapathy and Kalyansundarm (1988) studied on distribution of rice seed mycoflora within the grain with especial reference to storage fungi. Storage fungi found to be invading rice and remain mainly in the husk and outer layers of the kernel. The fungi invading the peripheral layer were mainly *Aspergillus flavus* and *A. nidulans* with *A. niger* to some extent. The more common ones being *A. candidus, A. glaucus* and sometimes *A. versicolor, Penicillium* spp. were less common there.

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

Mian and Fakir (1989) studied the occurrence of fungi associated with rough rice grains in the stored seeds of vars. Latishail and Naizersail determine relationship between germinability and associated seed borne fungi. A positive correlation between increase in storage fungi and loss in germinability was observed. The most predominant fungi in order of prevalence were *Helminthosporium oryzae, Curvularia lunata, Cladosporium cladosporioides, Aspergillus* spp. and *Trichoconis padwickii.*

Fakir *et al.* (1990) detected seed borne fungal pathogen of rice seed in Bangladesh; these were *Curvularia lunata, Drechslera oryzae, Fusarium* spp., *F. moniliforme, Phoma* sp. and *Trichoconis padwickii.* Among these, *F. moniliforme* was found to be the most prevalent occurring in 58 and 59 seed sample of Pajam and Mala, respectively out of 60 sample of each of the two varieties. As high as 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 national seed health standard fixed for those pathogens. Average germination of most of the seed samples was below 80%, which are lower then the national germination standard.

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 and 6 bacterial). The disease were brown spot *(Bipolaris oryzae),* stack burn *(Alternaria padwickii)* leaf scald *(Rhynchosporium oryzae),* bakanae disease and foot rot *(Fusarium moniliforme/Gibebrella fujikuroi),* sheath blight *(Rhizoctonia solani),* sheath rot *(Sarocladium oryzae),* false smut *(Ustilaginoidea virens),* kernel smut *(Tilletia barclayana),* 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).*

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

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

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

Roy (1993) observed that *Curvularia lunata* was the most common (37%) fungi associated with discoloured grains, followed by *Fusarium* spp. (13%) and *Chaetomium* (6%).

Wu and Dow (1993) examined 55 rice seed samples collected from major rice growing areas in Taiwan. Among the 25 fungi detected by blotter and deep-freeze methods, *Ascochyta oryzae, Acremoniella atra, Alternaria tenuis (A. alternata), Curvularia affinis, C. brachyspora, C. clavala, C. [Cochliobolus] pallescens, Diplodia oryzae, Drechslera*

halodes (Setosphaeria rostrata), D. poae, D. rostrota. Fusarium lateritium (Gibberella baccata), F. semitectum (F. pallidoroseum), Rhynchosporium oryzae, (Monographella albescens) and *Trichoconis (Alternaria) padwickii* were isolated from rice for the first time in Taiwan. *Phoma glumarum* and *M. albescens* occurred more frequently on seed harvested from northern and eastern Taiwan than from central a»d southern areas, *Curvularia lunata [Cochliobolus lunatus]* and *A. padwickii* more often from central and southern Taiwan.

Bhuiyan *el al.* (1994) detected the incidence of *Pyricularia oryzae* in 28 seed samples of rice out of 173 samples tested. The highest incidence in individual sample recorded was 18.0%. The incidence of the fungus was found more in unfilled grains compared to filled grain.

Bhutta and Ahmed (1994) collected 153 rice seed samples from paddy crop growing areas in Pakistan and tested for the presence of seed borne bacterial pathogen using seedling symptoms test. Maximum seed infection due to *Xanthomonas oryzae* pv. *oryzae* was 11% and 12% in variety IRRI-6 at Lahore and Hyderabad, respectively. *Acedovorax avenae* pv. *avenae* infection was 13% in variety B-385 from Sahiwal. Percentage seed infection due to bacterial pathogens varied from cultivar to cultivar in different localities.

Sisterna *et al.* (1994) isolated *Curvularia lunata, C. protuberata, Bipolaris oryzae, Epicoccum* spp., *Alternaria* sp. *Fusarium semitectum, F. equiseti, F. graminearum, F. oxysporum* and an unidentified species from 9 rice seed sample with black dots, discoloration, chalky spots and other symptoms from 2 provinces in Argentina during 1988-1989.

Islam *et al.* (1994) conducted a survey on seed borne pathogens of rice in fifteen districts of Bangladesh. They examined seed health of 83 samples of rice collected from 15 district of Bangladesh tested by the blotter method of testing and found 7 fungal pathogens associated with rice grains. Incidence of these pathogens was found to vary with respect to location and source of collection. Farmer's seeds, in general, were infected more than those from Government farms. However, seed from Tabunia Farm, Pabna were heavily infected. Average incidence of *Drechslera oryzae* and *Trichoconis padwickii (Alternaria padwickii)* was much, higher in the north of the country compared to the south.

Misra *et al.* (1994) screened 144 rice seed samples collected from 7 different regions of the Philippines 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 *Nakatea sigmoideum* were evenly distributed during the dry season. During the wet season, distribution of *Drechslera sp.* and *Microdochium oryzae* was even. Infection of both apparently healthy and discolored seeds was highest with *Alternaria padwickii* followed by *Curvularia* sp.

Ilyas and Javid (1995), Out of 46 samples 30 yielded *Fusarium moniliformae (Gibberella fujikuroi),* 45 *Alternaria padwickii,* 7 *Alternaria longissima,* 41 *Drechslara oryzae,* 2 *Phoma* sp. and 1 each *Curvularia oryzae* and *Cercospora* sp.

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, followed by *Curvularia, Fusarium* and *Aspergillus* spp.

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

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

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

Disthaporn *et al.*(1998) in Thailand assessed on farm seed management practices in rice *Alternaria padwickii, Curvularia lunata (Cochliobolus lunata)* were the major pathogen of seeds. All the seed samples were below the standard seed quality for growing purpose.

Bicca *et al* (1998) conducted the study with rice seed following blotter method. Fungi observed in 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.

Khan *et al.* (1999) isolated various fungi viz, *Fusarium moniliforme (Gibberella fujikoroi), F. semitectum (F. pallidoroseum), F. oxysporum, Alternaria alternata, A. padwickii, Curvularia oryzae, C. lunata (Cochliobolus lunata), Drechslera oryzae [Cochliobolus miyabeanus], Pyricularia oryzae [Magnaporthe grisea]* and species of *Nigrospora, Phoma, Aspergillus* and *Penicillium* from 38 rice samples of 16 different varieties/lines.

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 *(B. oryzae, Sclerotium rolfsii* and *Fusarium* spp.) and grain spot *(Curvularia lunata, Nigrospora oryzae, Phoma glumarum, Cladosporium* sp.).

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. BR11. 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.

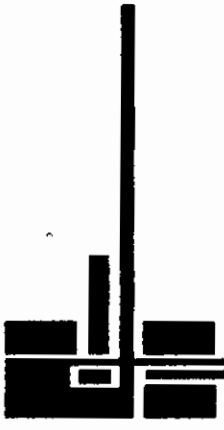
Islam *et al.* (2000) conducted and experiment with nine seed samples of rice cv. BR11 collected from farmer's storage and analyzed for *B. oryzae* incidence using the blotter method. Incidence of *B. oryzae, Trichoconis padwickii, Curvularia lunata, Aspergillus* spp. *and Penicillium* spp. ranged from 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.

Tasleem-uz-Zaman *et al.* (2000) reported that rice seed sample yielded *Fusarium moniliforme (Gibberella fujikuroi), F. semitectum [F. pallidoroseum], Alternaria padwickii, Alternaria miyabeanus* and *Aspergillus niger.*

Naeem Khalid *et al* (2001) determined the incidence of mycoflora, their frequency and impact on germination of four different rice cultivars. They reported five storage fungi viz. *Aspergillus flavus, Aspergillus niger, Chaetomium globosum, Penicillium* spp., and *Rhizopus stolonifer [R. stolonifer* var. *stolonifer]* were associated with rice seeds. The associated mycoflora reduced the seed germination of all the cultivars.

Fakir *et al.* (2002) determine the quality of farmers' saved rice seeds of Rajshahi, Rangpur and Bogra in Bangladesh before sowing. Total number of 354 rice seed samples was collected from farmer's storage of three

different locations (Bogra, Rajshahi and Rangpur) and determined the quality of farmers' saved seeds. Moisture content of seed ranged from 7.0 to 13.9% varying with respect to crop seasons, farmers and locations of seed collection. All the contaminants viz. rice varietal mixture (1.20-5.91%), other plant parts (0.01-0.17%), inert matter (0.09-0.20), partially filled seed (1.00-2.54) and unfilled seed (0.06-2.73%) were in the farmers saved rice seeds. Three species of insects namely rice moth (0.09-187.66%), rice weevil (0.03-9.94%) and red flour beetle (2.00-46%) were encountered in rice seeds. Six types of abnormal seed viz. discolored seed (14.43-24.44%), spotted seed (33.72-37.71%), deformed seed (8.46-15.50%), insect damaged seed (0.03-0.90%), germinated seed (0.0002-0.008%) and smutted seed (0.0001-0.017%) were recorded where deformed seeds had relatively higher occurrence (15.50%) and amount of pure seeds (91.20-98.89%) and best seed (19.56-63.65%)were recorded. Also five important pathogenic fungi viz. *Alternaria padwickii, Fusarium moniliforme, Bipolaris oryzae, Pyricularia oryzae and Sarocladium oryzae* were detected in rice seed samples varied in prevalence with respect to crop season and sites of seed collection.



Chapter 3
Materials and Methods

CHAPTER 3

MATERIALS AND METHODS

3.1. Experimental Site

The experiment was conducted at the Seed Pathology Laboratory and Plant Disease Diagnostic Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Dhaka.

3.2. Experimental Period

The experiment was conducted during the period from November, 2008 to April, 2009.

3.3. Collection of seed samples

Altogether 15 seed samples hybrid rice varieties and two HYVs were collected from different seed importer, Bangladesh Rice Research Institute (BRRI) and local market of Bangladesh.

3.4. Rice varieties used in this experiment

Altogether 13 imported hybrids, 2 local hybrids and 2 local high yielding varieties were used in this experiment. Detailed information of the used hybrid varieties and check varieties are given in Table 1.

Table1. Detailed information of the used hybrid varieties and check Varieties of boro rice in the experiment

Sl. No.	Name of varieties	Line/Cross combination	Origin	Source of collection
1.	Hira-1	99-5	China (Imported)	Supreem Seed Co. Ltd.
2.	Hira-2	HS-273	China (Imported)	Supreem Seed Co. Ltd.
3.	ACI 1	F1 Seed	China (Imported)	ACI Limited
4.	Surma 1	F1 Seed	China (Imported)	Syngenta Bangladesh Ltd.
5.	Taj-1	GRA2/06	China (Imported)	National Seed Co. Ltd.
6.	Modumoti-2	WBR-2	China (Imported)	United Seed Co. Ltd.
7.	Krishan-2	S-2B/07	China (Imported)	Mukterpur Bhandar
8.	Sonar bangla-6	HTM-6	China (Imported)	Mallika Seed Co. Ltd.
9.	Richer-101	R-101	China (Imported)	Lal Teer Seed Co. Ltd.
10.	Moyna	HTM 303	China (Imported)	Lal Teer Seed Co. Ltd.
11.	Tia	HTM 707	China (Imported)	Lal Teer Seed Co. Ltd.
12.	Tinpata	T-40	China (Imported)	Tinpata Quality Seed Bangladesh Ltd.
13.	Aloron	HB-8	China (Imported)	BRAC Seed Enterprise
14.	BRRI hybrid dhan-1	IR58025A x BR827R	Bangladesh (Local)	Bangladesh Rice Res. Inst.
15.	BRRI hybrid dhan-2	BRRI A x BR168R	Bangladesh (Local)	Bangladesh Rice Res. Inst.
16.	BR 28	check variety	Bangladesh (Local)	Bangladesh Rice Res. Inst.
17.	BR 29	check variety	Bangladesh (Local)	Bangladesh Rice Res. Inst.

3.5. Detection of seed borne pathogens

To identify the different pathogens on different hybrid rice varieties, the following three methods were used in this experiment:

3.5.1. Blotter Method

The collected seed samples of rice were analyzed for the presence of major seed borne fungal pathogens by blotter method following the International rules for Seed Testing (ISTA, 1996).Two hundred seeds were tested for each treatment maintaining four replications. Twenty-five seeds were placed on three layers of moist blotting paper (Whatman No.1) in each glass petridish. The petridishes were incubated at 25±1°C under 12/12 hrs light and darkness cycle for 7 days. Each seed was observed under strereomicroscope in order to record the presence of fungal colony and bacterial ooze 7 days after incubation based on growth habit. In doubtful cases temporary slides were prepared from the fungal colony and observed under compound microscope. Appropriate keys (Booth, 1971; Chidambaram *et al.*, 1973; Misra *et al.*, 1994 and Malone and Muskette1964.) were consulted for identification of the fungi and bacteria. The results were presented as percent incidence for individual pathogen. Germination of the seeds was also recorded.

3.5.1.1. Inspection of incubated seed samples

Each individual incubated seed was observed under stereomicroscope at 16x and 25x 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 Mathur and Kongsdal (2003).

Plate 1. Blotter Method

For proper identification of fungi temporary slides were prepared from the fungal colony and observed under compound microscope at 100x and 400x, and identified with the help of Keys suggested by Malone and Muskette (1964), Booth (1971), Ellis (1971), Chidambaram and Mathur (1975), Neergaard (1979).

The fungi from the incubated seeds were also transferred to PDA when needed. The culture was incubated at 25±10°c for 3-7 days. Temporary semi permanent slides prepared from the fungal colony and observed under compound microscope. The fungi were identified with the help of different books, manuals and publications (Benoti and Mathur, 1970; Ellis, 1971 and 1976: Agarwal *et al.*, 1989).The results were presented as percent incidence for individual pathogen.

3.5.2. Rolled paper towel method

The method developed by Sing and Rao (1982) was followed. Germinability of the seeds were determined in the laboratory at room temperature (30 ±2°C). 200 seeds were randomly taken from each variety and 40 seeds were placed between a pair of moist paper towels .There were four replications for each variety. The towels were rolled and the ends were closed by threads and covered by polyethylene paper to prevent drying. After 10 days of incubation period observations pertaining to (a) % germination, (b)Non germinated seed (hard seed and rotten seed), (c) Post-emergence death, (d) Shoot length (e) Root length (f) Vigor Index and (g) Incidence of different organism.

For determination of organisms some portion of the fungi growth on the infected seeds were taken with the needle and observed under compound microscope.

For determination of seedlings vigour 10 seedlings (normal /abnormal) were randomly selected from each paper and their individual shoot and root length was measured. Length of shoot was measured from the base of the stem up to the growing point of the youngest leaf. Similarly, length of root was measured from the starting point of the root to the largest available lateral root apex. Vigour of the seedling was determined by the following formula (Baki and Anderson, 1972)

Vigour Index = (mean of root length+ mean of shoot length) × percentage of seed germination.

Plate 2. Rolled paper towel method

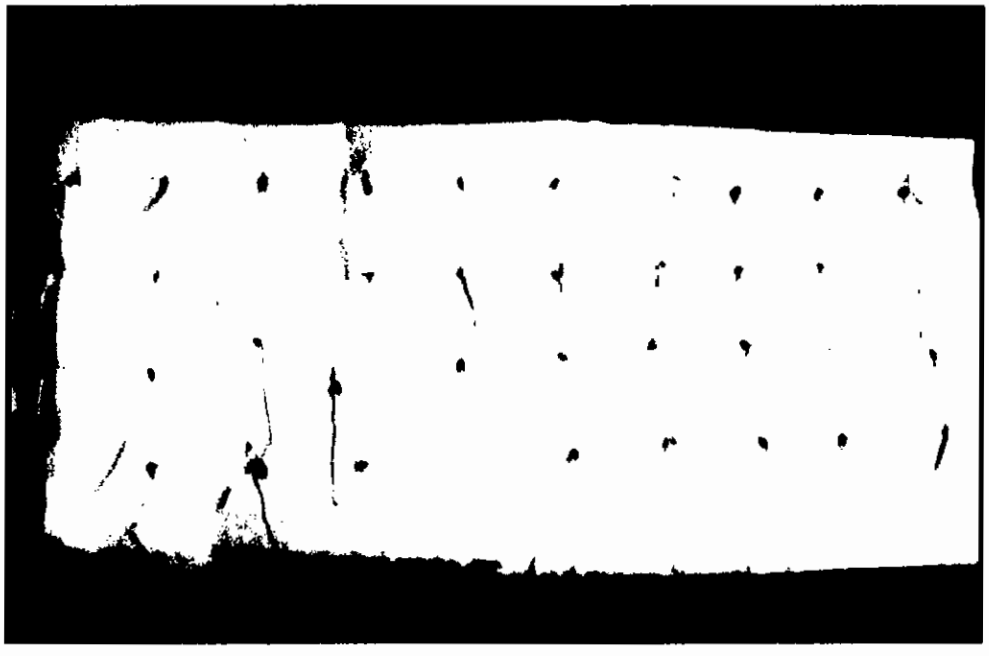


Plate 3. Ten days old seedlings in rolled paper towel method

3.5.3. Agar Plate Method

In the agar plate method, two hundred seeds were tested for each maintaining four replications. Surface disinfected seeds(0.1% mercuric chloride) were plated on the PDA medium and the plated seeds were usually incubated for 5-7 days at 22-25°C under 12h altering cycles of light and darkness. At the end of the incubation period, fungi growing out from the seeds on the agar medium were examined and identified. Identification was done based on colony characters and morphology of sporulation structures under a compound microscope. In the agar plate method more than one type of fungal colonies may be produced. In this case, identification was done on the most frequently occurring colony present in all the petridishes, and then the second most frequent, the third most frequent and so on. Thereafter, the identification of the different colonies were done visually and then under a stereomicroscope and followed by an examination of the fruiting structures under a compound microscope. Once the identification was done, the colonies were assigned names and their acronyms written on the reverse.(S.B. Mathur and Kongsdal,2003.)

Plate 4. Agar plate method

3.6. Preparation of culture media

3.6.1 Potato Dextrose Agar Medium

200g clean and healthy potato slice were taken in saucepan in 500ml water and boiled. The extract was filtered throw a fine cloth and then transferred in a clean beaker. 17g agar was melted in 500 ml of water in another beaker and the extract of potato was added to melt agar and was stirred with a glass rod. 20g of dextrose was dissolved and the volume was raised to 1000ml with distilled water. The mixture was agitated and heated with frequent stirring with a glass rod to dissolve and melted agar. The medium was poured to flask or tubes and was plugged with cotton and autoclaved for 15 minutes at 121°c under 15 PSI in an autoclave. After autoclaving, the liquid medium was poured in the petridishes and solidified.

3.6.2. Nutrient agar medium (NA)

Nutrient agar (15g) was taken in the Erlenmeyer flask containing 1000 ml distilled water. Peptone (5g) and beef extract (3g) were added to flask. For mixing properly the nutrient agar was shaked thoroughly for few minutes. Flask was then plugged with cotton and wrapped with a piece of brown paper and tied with thread. It was then autoclaved at 121°c under 15 lbs pressure for 15 minutes. After autoclaving, the liquid medium was poured in the petridishes and solidified.

3.7. Isolation of fungi

Seed borne fungi were isolated from the infected rice samples and observed under microscope and made pure cultures of them in potato dextrose agar media.

3.8. Isolation and identification of bacteria

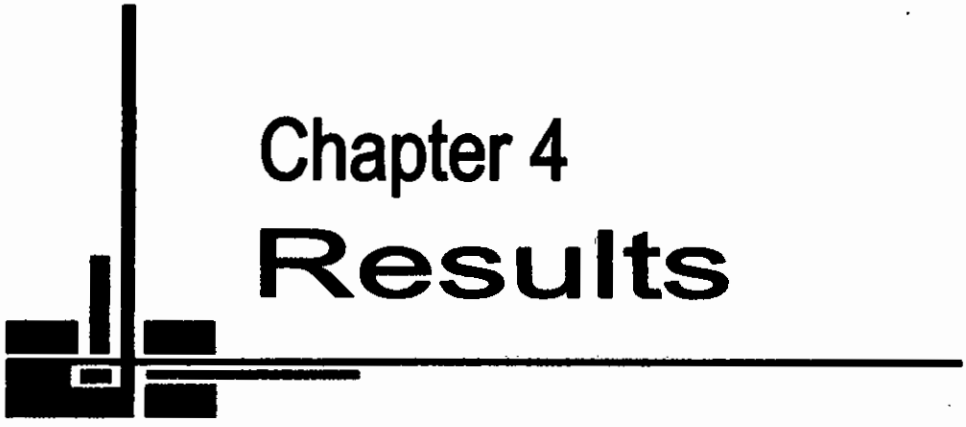
The bacterial ooze comes out from the seeds were collected. Then the needle was soaked in ooze and the needle was again soaked with sterile water in a test tube and makes a lower concentrated ooze solution. The bacterial ooze was streaked out with a wire loop on to the nutrient agar plate from the test tube containing low concentrated bacterial ooze. Then these plates were placed in the incubator for 24-36 hours. Bacterial colony, light yellow colour was seen in media and *Xanthomonas oryzae* was identified by physiological study; Gram reaction test.

The procedure of Gram staining (Hucker's modification) -A drop of diluted inocula suspension taken by sterilized needle was placed on the slide and was fixed with the least amount of heat. Then the slide was immersed in iodine solution for one minute and washed in tap water. The water was shaken off from the slide but was not allowed to dry. The slide was decolorized for 25 seconds with 95% ethyl alcohol and then washed thoroughly in tap water and shake off excess water. Finally the slide was counterstained with safranine for 15-20 seconds and washed in tap water. The slide was dried by blotting paper or air dry and observed under the compound microscope.

Gram negative bacteria appear red colour after decolourization with 95% ethyl alcohol and gram positive ones violet colour

3.9. Design of the experiment

The laboratory experiment was conducted following Completely Randomized Design (CRD) with four replications. The recorded data on various parameters under the present study were statistically analyzed using MSTAT statistical package.



Chapter 4
Results

CHAPTER 4

RESULTS

4.1. Determination of seed health status by blotter method

4.1.1. Percent seed germination

Germination records of different hybrids and high yielding varieties of rice investigated through blotter methods. The highest seed germination was found in Hira-2 (99.50%) followed by Modhumoti-2 (95.50%), ACI-1(94.00%), Hira-1(90.25%), respectively. The lower germination was recorded in BRRI hybrid dhan-2 (54.63%) preceded by BRRI hybrid dhan-1(63.25%), Sonar Bangla-6 (74.50%) and Moyna (83.00%) which was statistically identical with Richer-101(82.00%) and Taj-1 (82.75%) (Table-2).

4.1.2. Identified Pathogens

The identified pathogens were *Xanthomonas spp,, Rhizopus stolonifer, Aspergillus* spp., *Fusarium moniliforme, Phoma sp., Bipolaris oryzae, Curvularia lunata, Penicillium* sp., *Alternaria tenuissima, Nigrospora* sp, *Chaetomium globosum* and *Tilletia barclayana.*

4.1.3. Incidence of seed borne pathogens

Results regarding the incidence of *Xanthomonas* spp. of different hybrids and High yielding varities of rice shown in Table-2. The incidence of *Xanthomonas* spp. of different hybrids and HYV were significantly different from one to another that ranged from 0.00 to 18.13%. The highest incidence of *Xanthomonas* spp. was observed on

Sonar Bangla-6 (18.13%) which was statistically identical with Tinpata (17.50%). There was no incidence observed on Hira-1 and Hira-2. The lower incidence was found on Aloron (0.25%) which was statistically similar with BRRI hybrid dhan-1 and BRRI dhan 29. The rest of the varieties showed the medium incidence.

The incidence of *Rhizopus stolonifer* ranged from 0.00 19.75%. The highest incidence was observed on BRRI hybrid dhan-2 (19.75%) followed by Taj-1(9.25%), Moyna (7.00%) and Tia (6.75%). No incidence was observed on Hira-2. The lower incidence was observed on Hira-1 (0.38%) which was statistically identical with ACI-1, Surma-1, Modhumoti-2, Krishan-2, Sonar Bangla-6, Richer-101, Tinpata, Aloron, BRRI dhan-29 and BRRI dhan-28. (Table-2).

The incidence of *Aspergillus* spp. ranged from 0.00 to12.00% .The highest incidence was observed on Hira-2 (12.00%) followed by Moyna (3.25%). No incidence was observed on Hira-1, Raicher-101, Tia, Tinpata and Aloron. The rest of the varieties showed the medium incidence (Table-2).

The incidence of *Fusarium moniliforme* ranged from 0.00 to 9.50%. The highest incidence was observed on ACI-1 (9.50%). No incidence was found on Surma-1, Sonar Bangla-6, Raicher-101, Moyna, Tia, Tinpata, Aloron, BRRI hybrid dhan-2 and BRRI dhan-29. The lower incidence was found in Hira-1(1.00%) and BRRI hybrid dhan-1(1.00%) which were statistically identical with Krishan-2 and BRRI dhan-28 (Table-2).

The incidence of *Phoma* sp. was observed only on Taj-1.The incidence was 0.50% (Table-2).

The incidence of *Bipolaris oryzae* differ significantly from each other that ranged from 0.00 to 18.00%. The highest incidence was observed on Aloron (18.00%) followed by Surma-1(3.75%), Modhumoti-2 (3.50%) and Hira-1 (3.25%). No incidence was found in Hira-2, ACI-1, Taj-1, Krishan-2, SonarBangla-6, Raicher-101, Moyna, Tia, Tinpata, Aloron and BRRI hybrid dhan-2. The lower incidence was observed on BRRI dhan-28 (0.25%) preceeded by BRRI hybrid dhan-29 (1.50%) (Table-2).

The incidence of *Curvularia lunata* ranged from 0.00 to 4.25%. The highest incidence was observed on Aloron (4.25%) closely followed by ACI-1(2.00%). The lower incidence was observed on BRRI dhan-28 (0.25%). Only 0.50% seed infection observed on Modhumoti-2, Krishan-2, Tia, Tinpata and BRRI hybrid dhan-2. The rest of the variety did not showed any seed infection.

The incidence of *Penicillium* sp. ranged from 0.00 to 1.75%. The highest incidence was observed on Modhumoti-2 (1.75%) closely followed by BRRI hybrid dhan-1(1.00%), BRRI dhan-28(1.00%). No incidence was observed on Hira-1, Hira-2, ACI-1, surma-1, Taj-1, Sonar Bangla-6, Richer-101, Moyna, Tia, BRRI hybrid dhan-2. The lower incidence was found on Tinpata, Aloron (0.25%) preceded by Krishan-2(0.50%) (Table-2).

The incidence of *Alternaria tenuissima* ranged from 0.00 to0.63%.The highest incidence was observed on Hira-1(0.63%) followed by Aloron(0.50%), Tinpata (0.25%), Modhumoti-2 (0.25%). The rest of the varieties showed no seed infection.

The incidence of *Nigrospora* sp was observed only on Hira-1. The incidence was 0.25% (Table-2).

The incidence of *Chaetomium globosum* was observed only on Modhumoti-2. The incidence was 1.25% (Table-2).

The incidence of *Tilletia barclyana* was observed only on Richer-101. The incidence was 2.75% (Table-2).

4.1.4. Frequency and occurrence of various seed borne pathogens

The frequency and occurrence of various pathogens recorded on different hybrids and Varieties by blotter method was presented in Table-3. Out of 2303 seed borne pathogenic infections recorded from 17 rice Varieties during the entire study, eleven fungi and one bacterium genera were identified. Of all these pathogens most predominant pathogen was *Xanthomonas* spp. (39.95%). Other predominant pathogens were *Rhizophus stolonifer, Aspergillus* spp., *Bipolaris oryzae, Fusarium moniliforme,*which constituted 23.31%, 11.07%, 10.94% and 7.29% of the total seed borne infections, respectively. *Nigrospora* sp had the lowest occurrence. Again, as regard to percentage of seed yielding individual organisms, 6.76% of the seed yielded *Xanthomonas* spp. followed by *Rhizophus stolonifer* (3.95%), *Aspergillus* spp. (1.88%), *Bipolaris oryza* (1.85%) and *Fusarium moniliforme* (1.23%).

15 rice varieties were found to be infected by *Xanthomonas* spp. and *Rhizophus stolonifer.* On the other hand, *Aspergillus* spp. infected 12 rice varieties. *Fusarium moniliforme, Curvularia lunata* infected 8 rice varieties. *Bipolaris oryzae, Alternaria tenuissima, Penicillium* sp had infection in 6,4 and 4 rice Varieties, respectively. It was also found that 4

different rice Varieties were infected by *Nigrospora* sp., *Phoma* sp, *Chaetomium globosum, Tilletia barclayana* (Table 3).

Table 2: Percent seed germination and incidence of different seed borne pathogens on imported hybrids and high yielding varities of rice seed by blotter method conducted during 2008-09

Hybrids and High yielding varities	% Seed germination	%Pathogen incidence											
		Xanthomons spp.	*Rhizopus stolonifer*	*Aspergillus* Spp.	*Fusarium moniliforme*	*Phoma* sp.	*Bipolaris oryzae*	*Curvularia lunata*	*Penicillium* Sp.	*Alternaria tenuissima*	*Nigrospora* sp.	*Chaetomium globosum*	*Tilletia barclayana*
Hira-1	90.25 b-d	0.00 e	0.38 e	0.00 c	1.00 de	0.00 b	3.25 b-d	0.00 c	0.00 b	0.63 a	0.25a	0.00 b	0.00 b
Hira-2	99.50 a	0.00 e	0.00 e	12.00 a	3.00 b	0.00 b	0.00 e	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
ACI-1	94.00 bc	1.75 c-e	1.50 e	0.25 c	9.50 a	0.00 b	0.00 e	2.00 b	0.00 b	0.25ab	0.00 b	0.00 b	0.00 b
Surma-1	90.25 b-d	4.25 c-e	1.25 e	1.50 bc	0.00 e	0.00 b	3.75 b	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
Taj-1	82.75 f	4.75 cd	9.25 b	1.63bc	2.50 bc	0.50 a	0.00 e	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
Modhumoti-2	95.50 ab	2.50 c-e	2.00 e	2.50 bc	1.50 cd	0.00 b	3.50 bc	0.50 c	1.75 a	0.25ab	0.00 b	1.25 a	0.00 b
Krishan-2	86.00d-f	6.00 c	3.63 c-e	1.75 bc	1.25 c-e	0.00 b	0.00 e	0.50 c	0.50 b	0.00 b	0.00 b	0.00 b	0.00 b
Sonar Bangla-6	74.50 g	18.13 a	0.50 e	0.25 c	0.00 e	0.00 b	0.00 e	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
Richer-101	82.00 f	14.75 ab	3.50 c-e	0.00 c	0.00 e	0.00 b	0.00 e	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	2.75 a
Moyna	83.00 f	14.00 ab	7.00 bc	3.25 b	0.00 e	0.00 b	0.00 e	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
Tia	84.25 ef	11.63 b	6.75 bc	0.00 c	0.00 e	0.00 b	0.00 e	0.50 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
Tinpata	87.75 df	17.50 a	0.25 e	0.00 c	0.00 e	0.00 b	0.00 e	0.50 c	0.25 b	0.25ab	0.00 b	0.00 b	0.00 b
Aloron	86.75d-f	0.25 e	2.75 de	0.00 c	0.00 e	0.00 b	18.00a	4.25 a	0.25 b	0.50ab	0.00 b	0.00 b	0.00 b
BRRI hybrid dhan-1	63.25 h	0.75 de	19.75 a	2.00 bc	1.00 de	0.00 b	1.25 c	0.00 c	1.00ab	0.00 b	0.00 b	0.00 b	0.00 b
BRRI hybrid dhan-2	54.63 i	10.63 b	5.86 b-d	0.50 c	0.00 e	0.00 b	0.00 e	0.50 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
BRRI dhan-28	89.00 c-e	5.50 c	0.50 e	1.50 bc	1.25 c-e	0.00 b	0.25 e	0.25 c	1.00ab	0.00 b	0.00 b	0.25 b	0.00 b
BRRI-dhan-29	89.50 c-e	1.00 de	1.25 e	0.75 c	0.00 e	0.00 b	1.50 cde	0.00 c	0.00 b	0.00 b	0.00 b	0.00 b	0.00 b
LSD $_{0.05}$	5.83	5.37	4.793	2.914	1.477	0.238	2.149	0.531	1.054	0.536	0.114	0.983	1.716
CV%	6.28	4.16	5.46	4.88	3.99	2.26	2.43	3.89	1.45	2.79	1.83	1.56	1.79

Values with different letters within a column differ significantly at 5% level of significance as per DMRT.

Table 3. Frequency and occurrence of various pathogens recorded on different imported hybrids and high yielding varieties of rice seed by blotter method conducted during 2008-09

Pathogens	No.of pathogenic infection	% of total infection[a]	% of the seed yielding[b]	No of Varieties infected[c]
Xanthomonas oryzae[d]	920	39.95	6.76	15
Bipolaris oryzae[d]	252	10.94	1.85	6
Rhizophus stolonifer[d]	537	23.31	3.95	15
Fusarium moniliforme[d]	168	7.29	1.23	8
Alternaria tenuissima	13	0.56	0.10	4
Nigrospora sp	2	0.086	0.02	1
Aspergillus spp[d]	255	11.07	1.88	12
Curvularia lunata	72	3.13	0.53	8
Phoma sp.	4	0.17	0.03	1
Chaetomium globosum	10	0.43	0.07	1
Penicillium sp	32	1.39	0.24	4
Tilletia braclayana	38	1.65	0.28	1

[a]Total number of seed borne pathoganic infections recorded on seeds of rice were 2303[b]

[b]Percentage of seed yielding different pathogens was calculated on the basis of 13600seeds

[c]Total number of seed samples were 17.

[d]Predominant pathogens constituted at least 5.0% of the total seed borne infections

Plate 5. Bacterial ooze in blotter method

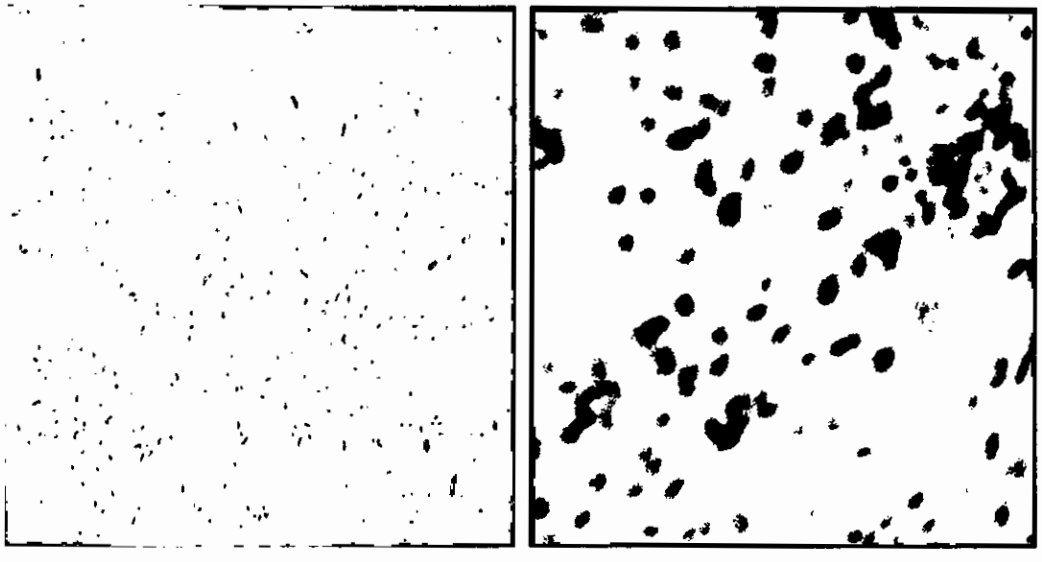


Plate 6. Cells of *Xanthomonas oryzae* (100x) and (400x)

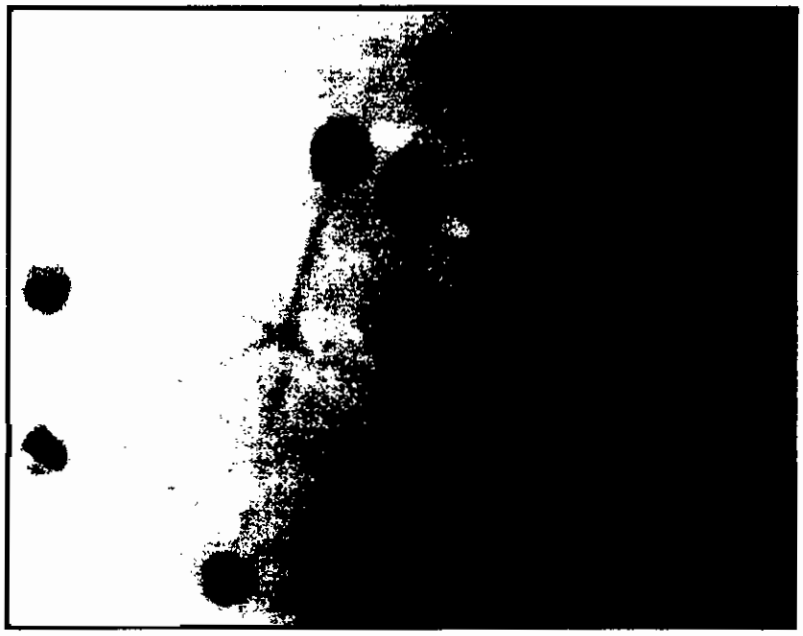


Plate 7. *Rhozopus stolonifer* under sterio microscope

Plate 8. *Rhozopus stolonifer* under Compound microscope (400x)

Plate 9. *Aspergillus flavus* under sterio microsope

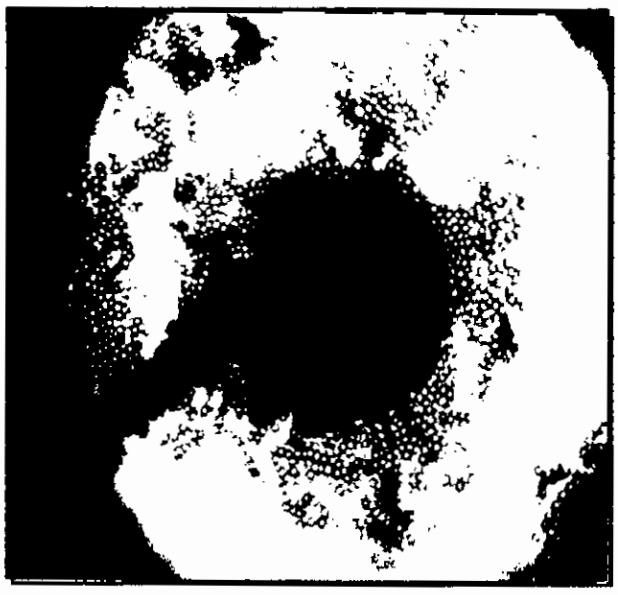


Plate 10.Aspergillus *flavus* under compound microscope (400 x)

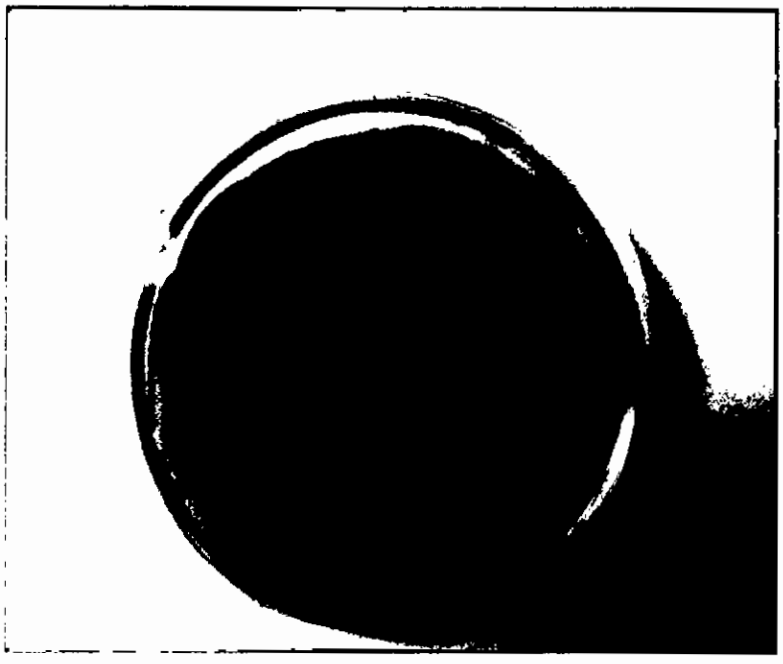


Plate 11.Pure culture of *Aspergillus flavus*

Plate 12. *Fusarium moniliforme* under compound microscope (100 x)

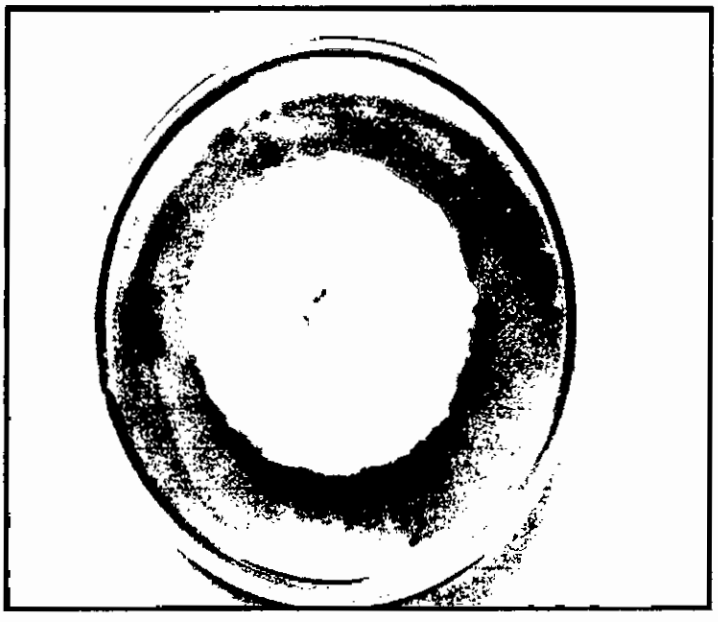


Plate 13. Pure culture of *Fusarium moniliforme*

Plate 14. *Penicillum* sp under compound microscope (100X)

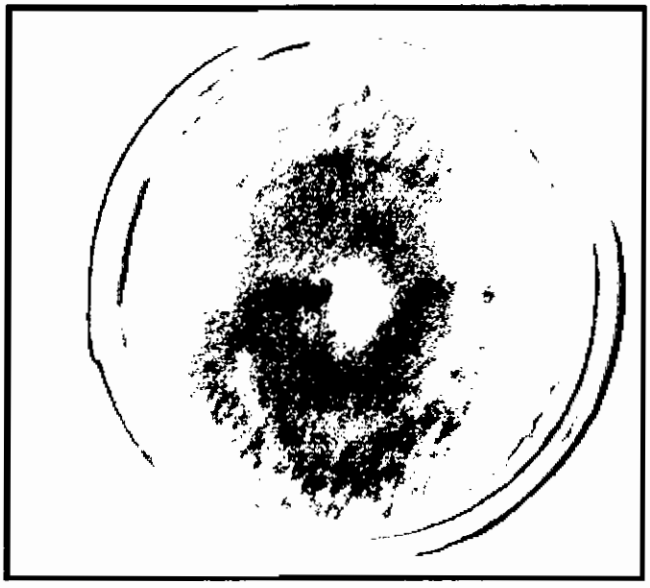


Plate 15.Pure culture of *Penicillum* sp

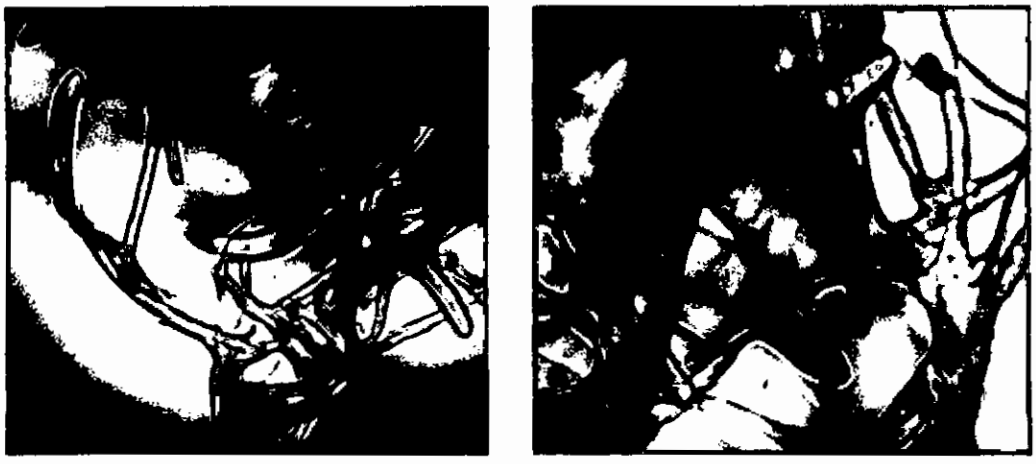


Plate 16. *Bipolaris oryzae* under compound microscope (100x &400X)

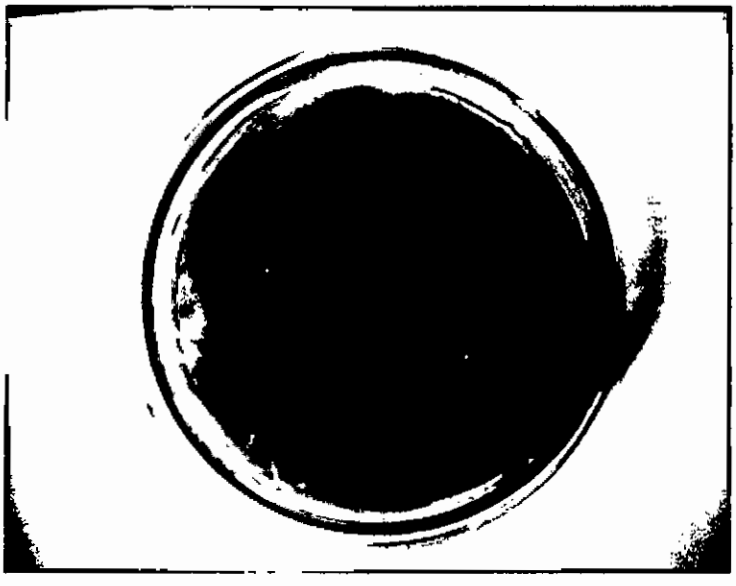


Plate 17. Pure culture of *Bipolaris oryzae*

Plate 18. ***Curvularia lunata*** **under compound microscope (400x)**

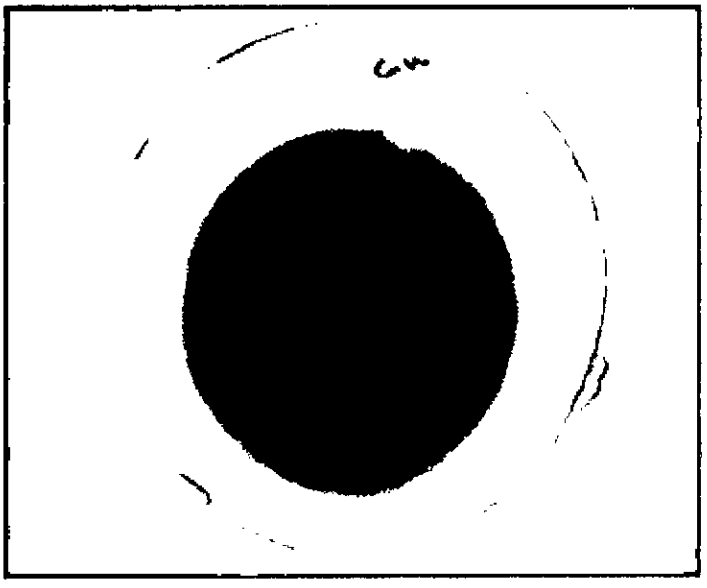


Plate 19. Pure culture of ***Curvularia lunata***

Plate 20. ***Alternaria tenuissima*** **under compound microscope (400X)**

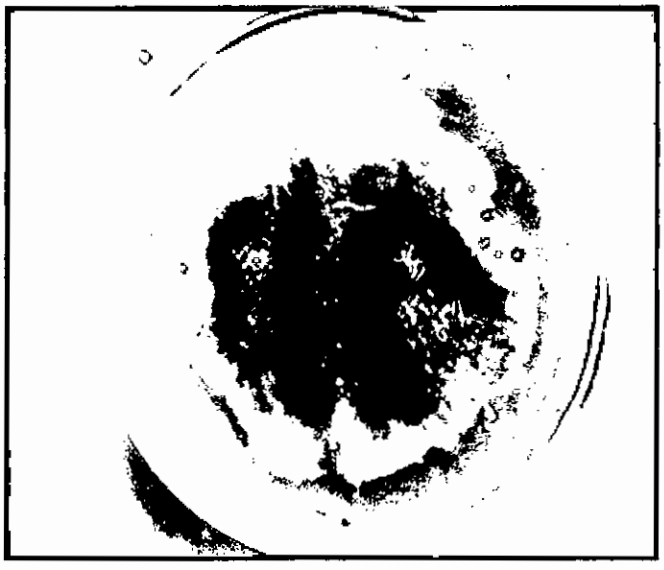


Plate 21. Pure culture of ***Alternaria tenuissima***

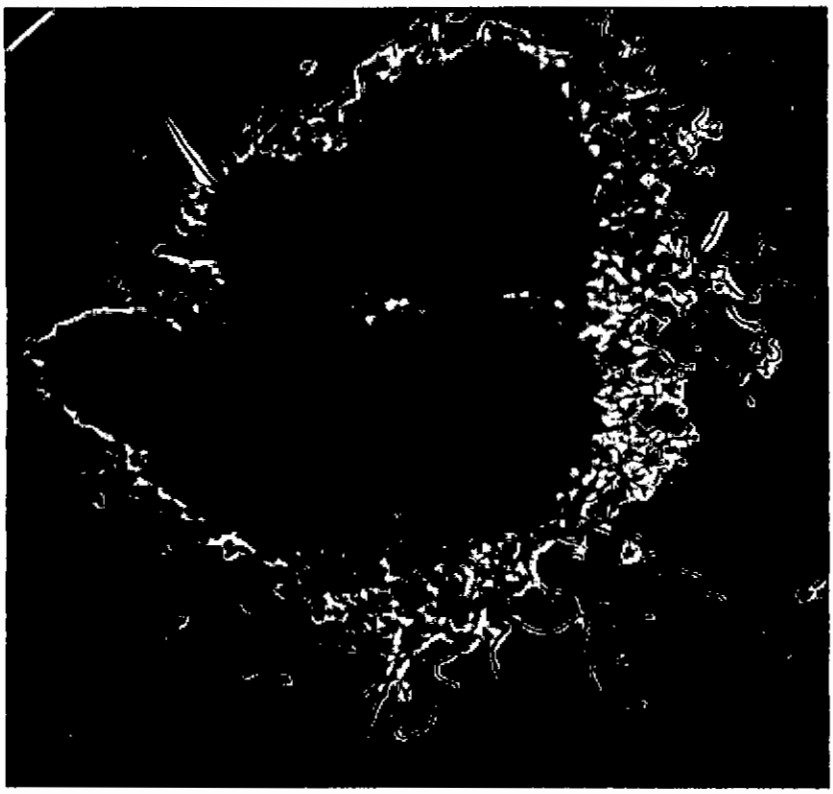


Plate 22. *Chaetomium globosum* under compound microscope (100x)

Plate 23. *Chaetomium globosum* under compound microscope (400x)

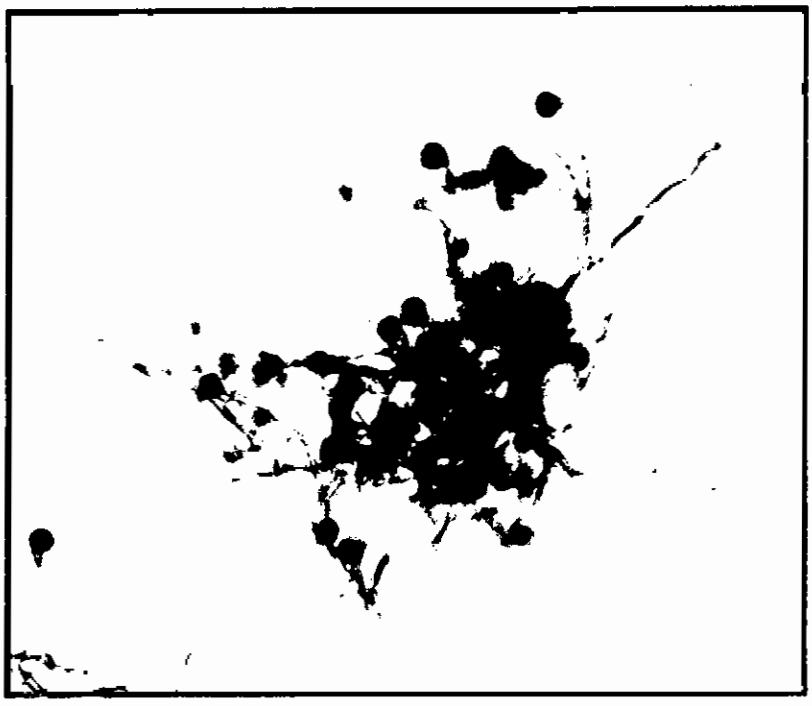


Plate24. *Nigrospora* sp. under compound microscope (100x)

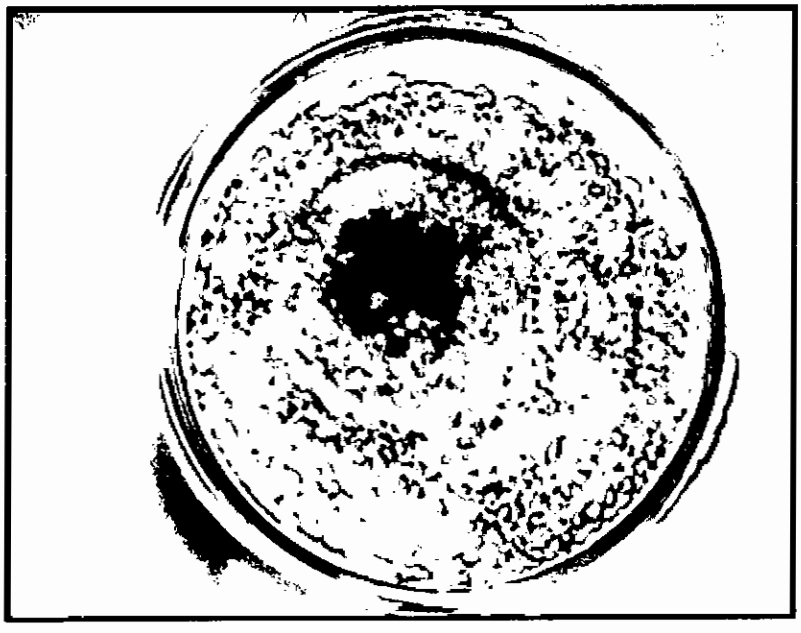


Plate 25. Pure culture of *Nigrospora* sp

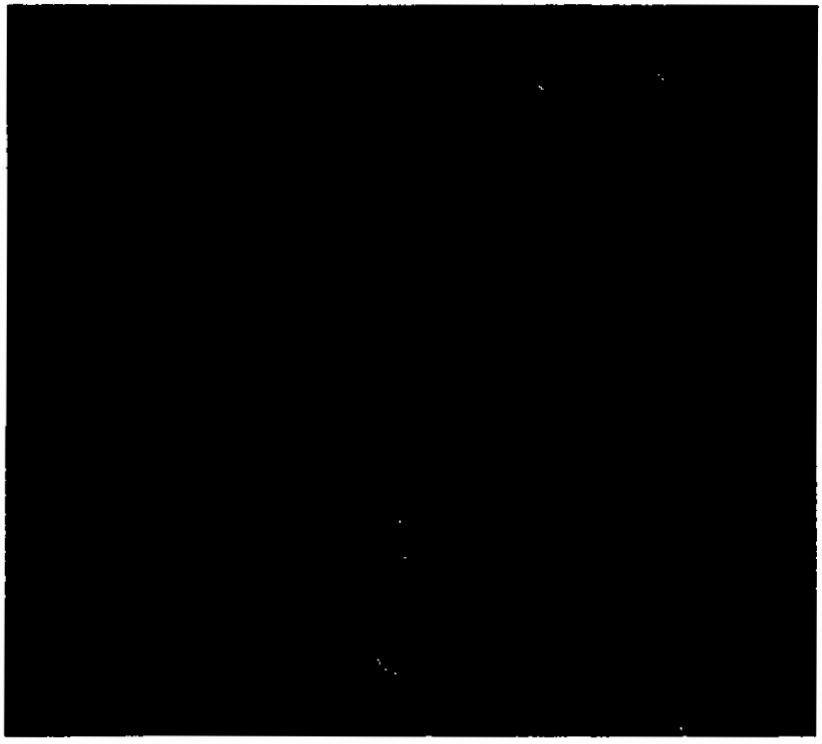


Plate 26. *Tilletia braclayan* under compound microscope (400X)

Plate 27. Pycnidia and pycnidiospore of *Phoma* sp. (100x)

4.1. Determination of seed health status by rolled paper towel method

4.2.1. Percent seed germination

Germination records of different hybrids and high yielding varieties of rice investigated through rolled paper towel methods. Percent seed germination differ significantly from one to another that ranged 8.25%-96.38%. The highest seed germination was found in Hira-1 (96.38%) which was statistically similar with Hira-2 (93.75%). The lowest germination was recorded in BRRI hybrid dhan-2 (8.25%) preceded by Taj-1(20.88%), Sonar bangla-6 (37.25%) (Table 4).

4.2.2. Non germinated (hard and rotten seed) and post-emergence mortality of different hybrids and high yielding varieties of rice by rolled paper towel method

Non germinated seed includes hard seed and rotten seed. Percentage of hard seed ranged from 1.63% to 86.50%. The highest percentage of hard seed was recorded on BRRI hybrid dhan-2 (86.50%) followed by Moyna (66.00%), Taj-1 (63.13%), Sonar Bangla-6 (51.25%).The minimum percentage of hard seed was recorded on ACI-1(1.63%) which was statistically identical with Modhumoti-2 (5.63%), Hira-1 (3.00%) and Hira-2 (2.00%) (Table-4).

Percentage of rotten seed ranged from 0.75 to 12.00. The highest percentage of rotten seed was recorded on Sonar Bangla-6 (12.00%) which was statistically identical with Tinpata (11.00%). The minimum percentage of rotten seed was recorded on Hira-2 (0.75%) (Table-4).

Percent Post-emergence mortality ranged from 0.00 to 4.38%. The highest percentage of post emergence death recorded on Aloron (4.38%). There was no post emergence mortality recorded on BRRI hybrid dhan-2

(0.00). The lower post emergence mortality was recorded on Krishan-2 which was statistically identical with Surma-1(0.75%), Taj-1(0.75%) (Table-4).

4.2.3.Seedling Vigour Index

Results of seedling vigour of 17 rice verities by paper towel method were analyzed and presented in Table 4. Vigour index ranged from 96.36 to 2329.28. Highest vigour index was observed in Hira-1 (2329.28) closely followed by Hira-2 (2168.32) which was statistically identical with ACI-1 (2165.60). The lowest vigour index was observed in BRRI Hybrid Dhan-2 (96.36) preceded by Taj-1 (310.75), Moyna (726.85). The highest root length was observed in Richer-101 (16.77cm) and the lowest was in BRRI Hybrid Dhan-2 (6.94cm). The highest shoot length was observed in Richer-101(11.38cm) and the lowest was in BRRI Hybrid Dhan-2 (4.75cm) (Table-4).

Table 4. Percent non germinated seeds, percent post emergence mortality and vigour index of different hybrids and high yielding varieties of rice seed recorded by rolled paper towel method conducted during 2008-09

Hybrids & High yielding varities	% Germination	%Non germinated seed		%Post-emergence mortality	Root length (cm)	Shoot length (cm)	Vigor index
		Hard	Rotten				
Hira – 1	96.38 a	3.00 j	0.75 g	2.25 ef	15.53 bc	8.73 e	2329.28 a
Hira – 2	93.75 ab	2.00 j	4.00 def	2.00 efg	14.68 c	8.46 e	2168.32 b
ACI– 1	91.75 b	1.63 j	6.25 b-d	3.88 ab	15.31 bc	8.39 e	2165.60 b
Surma – 1	63.25 h	33.25 e	3.25 e-g	0.75 h	10.68 g	6.02 h	1056.96 h
Taj – 1	20.88 k	63.13 b	5.50 c-e	0.75 h	9.30 h	5.13 i	310.75 j
Modhumoti – 2	91.63 b	5.63 j	2.88 e-g	3.63 bc	12.24 ef	7.06 g	1734.31 e
Krishan – 2	68.88 g	25.63 f	5.50 c-e	0.88 h	11.59 fg	7.28 fg	1296.92 g
Sonar Bangla – 6	37.25 j	51.25 c	12.00 a	1.88 efg	15.85 ab	9.91 c	977.46 h
Richer – 101	72.63 ef	18.75 h	9.13 ab	1.38 gh	16.77 a	11.38 a	2044.11 c
Moyna	73.13 ef	66.00 b	5.50 c-e	2.25 ef	14.90 bc	7.70 f	726.85 i
Tia	74.25 e	20.25 gh	8.00 bc	1.75 fg	14.98 bc	9.31 d	1798.21 de
Tinpata	70.13 fg	18.13 h	11.00 a	1.63 fg	15.63 bc	10.70 b	1843.73 d
Aloron	78.88 d	17.63 h	3.25 e-g	4.38 a	11.16 g	5.09 i	1280.77 g
BRRI hybrid dhan – 1	83.50 c	10.75 i	5.50 c-e	3.00 cd	13.11 de	8.58 e	1810.70 de
BRRI hybrid dhan – 2	8.250 l	86.50 a	5.25 c-e	0.00 i	6.94 i	4.75 i	96.36 k
BRRI dhan – 28	58.88 i	40.50 d	1.75 fg	3.13 cd	10.91 g	6.46 h	1048.49 h
BRRI dhan – 29	74.88 e	23.88 fg	1.25 fg	2.50 de	13.55 d	7.34 fg	1564.07 f
$LSD_{0.05}$	3.31	4.901	2.892	0.7235	1.028	0.5579	106.7
CV(%)	3.42	2.01	3.11	3.76	5.51	5.04	5.26

Values with different letters within a column differ significantly at 5% level of significance as per DMRT.

4. 3. Determination of seed health status by agar plate method

4.3.1. Identified pathogens

The identified pathogens were *Xanthomonas* spp., *Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger, Fusarium moniliforme, Bipolaris oryzae, Curvularia lunata, Penicillium* sp., *Alternaria padwickii, Nigrospora oryzae* .

4.3.2. Incidence of seed borne pathogens

Results regarding the incidence of *Xanthomonas* spp. of different hybrids and Varieties of rice shown in Table 5. The incidence of *Xanthomonas* spp. of different hybrids and varieties were significantly different from one to another that ranged from 1.63% to 15.13%. The highest incidence of *Xanthomonas* spp. was observed on Tinpata (15.00%) which was statistically identical with Tia (14.00%) and Sonar Bangla-6 (12.75%). The lowest incidence was observed on Hira-1(1.63%) which was statistically identical with Taj-1, Modhumoti-2, Aloron, BRRI hybrid dhan-1and BRRI dhan-29. The rest of the varieties showed the lower incidence.

The incidence of *Bipolaris oryzae* ranged from 0.00 to 15.13% (Table 5). The highest incidence was noticed on Aloron (15.13%). followed by Hira-1 (9.63%) and Surma-1 (5.00%). No incidence was observed on ACI-1, Taj-1, Krishan-2, SonarBangla-6, Raicher-101, Moyna, Tia, Tinpata and BRRI hybrid dhan-2. The lower incidence was found in BRRI hybrid dhan-28 (1.38%) preceeded by Hira-2 (2.13%).

The incidence of *Fusarium moniliforme* ranged from 0.00 to 7.63% (Table 5). The highest incidence was recorded on ACI-1 (7.63%)

followed by Hira-2 (3.88%). No incidence was observed on Surma-1, Sonar Bangla-6, Richer-101, Moyna, Tia, Tinpata, Aloron, BRRI hybrid dhan-2, BRRI dhan-29. The lower incidence was observed on BRRI dhan-28 (1.38%) preceded by Krishan-2 (2.75%) and Hira-1 (2.25%).

The incidence of *Rhizopus stolonifer* ranged from 0.00 to 3.00% (Table 5). The highest incidence of *Rhizopus stolonifer* was observed on Tia (3.00%) which was statistically identical with Richer-101, Moyna and BRRI hybrid dhan-1. No incidence was observed in Hira-2, Modhumoti-2. The lower incidence was noticed in Tinpata (1.00%) and Surma-1(1.00%).

The incidence of *Alternaria tenuissima* ranged from 0.00 to 1.63% (Table 5). The highest incidence was observed on Hira-1(1.63%) followed by Modhumoti-2 (1.50%), Tinpata (1.50%) and Krishan-2 (1.25%). The rest of the varieties had no incidence.

The incidence of *Nigrospora oryzae* was observed only on Hira-1. The incidence was 1.38% (Table 5).

The incidence of *Curvularia lunata* ranged from 0.00 to 6.38% (Table 5). The highest incidence was found on Aloron (6.38%) followed by ACI-1 (2.88%) which was statistically identical with Modhumoti-2 and Krishan-2. No incidence was observed in Hira-1, Hira-2, Surma-1, Taj-1, Richer-101, BRRI hybrid dhan-1, BRRI dhan-29.

The incidence of *Penicillium* sp. ranged from 0.00 to 1.88% (Table 5). The highest incidence was observed on BRRI hybrid dhan-1(1.88%) which was statistically identical with Modhumoti-2 (1.63%) and Krishan-2 (1.50%). The lower incidence was found in BRRI dhan-28 (0.88%)

which was statistically identical with Richer-101 (1.00%) and Tinpata (1.25%). Rest of varieties did not show any seed infection.

The incidence of *Aspergillus flavus* ranged from 0.00 to 6.50% (Table 5). The highest incidence was observed on Hira-2 (6.50%) followed by Taj-1(3.25%). No incidence was observed in Hira-1, Richer-101, Tia, Tinpata, Aloron. The lower incidence was found in Krishan-2 (1.13%).

The incidence of *Aspergillus niger* ranged from 0.00 to 1.38% (Table 5). The highest incidence was observed on Taj-1(1.38%) followed by Surma-1(1.13%), Modhumoti-2 (0.88%), Krishan-2 (0.63%). The rest of the varieties showed no incidence.

4.2.3. Frequency and occurrence of various seed borne pathogens by agar plate mehod

The frequency and occurrence of various pathogens recorded on different hybrids and high yielding varieties by agar plate method was presented in Table 6. Out of 2387 seed borne pathogenic infections recorded from 17 rice verities by Agar plate method. 10 fungi and 1 bacterium genera were identified. Of all these pathogens, most predominant organism was *Xanthomonas* spp. (40.51%). Rest predominant pathogens were *Bipolaris oryzae, Aspergillus* spp, *Fusarium moniliforme, Rhizophus stolonifer* and *Curvularia lunata* which constituted 15.63% 12.31% , 9.30%, 9.25% and 6.61% of the total seed borne infections, respectively. *Nigrospora* sp had the lowest (0.46%) occurrence. Again, as regard to percentage of seed yielding individual organisms 7.11% of the seed yielded *Xanthomonas* spp. followed by *Bipolaris oryzae* (2.74%), *Aspergillus* spp. (2.16%) *Fusarium*

moniliforme (1.63%) *Rhizophus stolonifer* (1.63%) *and Curvularia lunata* (1.16%).

All the 17 rice verities were found to be infected by *Xanthomonas* spp. On the other hand *Rhizopus stolonifer* infected 15 rice verities. *Bipolaris oryzae and Fusarium moniliforme* each infected 8 rice verities., *Aspergillus* spp., *Curvularia lunata, Penicillium* sp. *Alternaria tenuissima, Chaetomium globosum,* had infection in 12, 9 ,6, 4 and 2 rice Varieties, respectively . *Nigrospora* sp and *Tilletia barclayana* each infected 1 rice variety (Table 6).

Table 5. Incidence of different seed borne pathogens of imported hybrids and high yielding varieties of rice seed by agar plate method conducted during 2008-09

Hybrids and high yielding varieties	% Pathogen incidence									
	Xanthomonas spp.	*Bipolaris oryzae*	*Fusarium moniliforme*	*Rhizopus stolonifer*	*Alternaria tenuissima*	*Nigrospora oryzae*	*Curvularia lunata*	*Penicillium* sp.	*Aspergillus flavus*	*Aspergillus niger*
Hira – 1	1.63 f	9.63 b	2.25 cd	1.63 bc	1.63 a	1.38 a	0.00 f	0.00 d	0.00 d	0.00 e
Hira – 2	6.63 d	2.13 ef	3.88 b	0.00 d	0.00 c	0.00 b	0.00 f	0.00 d	6.50 a	0.00 e
ACI – 1	5.38 de	0.00 g	7.63 a	1.75 a-c	0.00 c	0.00 b	2.88 b	0.00 d	1.50 b-d	0.00 e
Surma – 1	4.50 de	5.00 c	0.00 e	1.00 cd	0.00 c	0.00 b	0.00 f	0.00 d	2.13 bc	1.13 b
Taj – 1	3.43 ef	0.00 g	3.63 bc	1.50 bc	0.00 c	0.00 b	0.00 f	0.00 d	3.25 b	1.38 a
Modhumoti – 2	3.25 ef	3.00 de	3.38 bc	0.00 d	1.50 ab	0.00 b	2.25 bc	1.63 ab	1.88 b-d	0.88 c
Krishan – 2	6.75 d	0.00 g	2.75 b-d	2.25 a-c	1.25 b	0.00 b	1.88 bc	1.50 ab	1.13 cd	0.63 d
Sonar Bangla – 6	12.75 a-c	0.00 g	0.00 e	1.50 bc	0.00 c	0.00 b	0.25 ef	0.00 d	2.75 bc	0.00 e
Richer – 101	11.50 bc	0.00 g	0.00 e	2.38 ab	0.00 c	0.00 b	0.00 f	1.00 c	0.00 d	0.00 e
Moyna	6.25 d	0.00 g	0.00 e	2.50 ab	0.00 c	0.00 b	1.25 c-e	0.00 d	2.63 bc	0.00 e
Tia	14.00 ab	0.00 g	0.00 e	3.00 a	0.00 c	0.00 b	0.75 d-f	0.00 d	0.00 d	0.00 e
Tinpata	15.00 a	0.00 g	0.00 e	1.00 cd	1.38 ab	0.00 b	1.25 c-e	1.25 bc	0.00 d	0.00 e
Aloron	3.38 ef	15.13 a	0.00 e	1.50 bc	0.00 c	0.00 b	6.38 a	0.00 d	0.00 d	0.00 e
BRRI hybrid dhan – 1	4.25 d-f	3.13 de	3.50 bc	2.44 ab	0.00 c	0.00 b	0.00 f	1.88 a	3.38 b	0.00 e
BRRI hybrid dhan – 2	10.13 c	0.00 g	0.00 e	1.88 a-c	0.00 c	0.00 b	1.63 cd	0.00 d	2.75 bc	0.00 e
BRRI dhan – 28	5.88 de	1.38 fg	1.50 d	1.50 bc	0.00 c	0.00 b	1.63 cd	0.88 c	2.00 bc	0.00 e
BRRI dhan – 29	4.38 d-f	4.13 cd	0.00 e	1.25 b-d	0.00 c	0.00 b	0.00 f	0.00 d	2.63 bc	0.00 e
$LSD_{0.05}$	2.786	1.394	1.403	1.361	0.3364	0.08992	1.024	0.4863	1.954	0.2203
CV(%)	4.99	3.32	5.87	3.13	2.10	4.97	6.85	1.63	1.87	6.12

Values with different letters within a column differ significantly at 5% level of significance as per DMRT.

Table 6. Frequency and occurrence of various pathogens recorded on different hybrids and high yielding varieties of rice seed by agar plate method (2008-09)

Pathogen	No.of pathogenic infection	% of total infection[a]	% of the seed yielding[b]	No of Varieties infected[c]
Xanthomonas oryzae[d]	967	40.51	7.11	17
Bipolaris oryzae[d]	373	15.63	2.74	8
Rhizophus stolonifer[d]	221	9.25	1.63	15
Fusarium moniliforme[d]	222	9.30	1.63	8
Alternaria tenuissima	46	1.93	0.33	4
Nigrospora sp	11	0.46	0.08	1
Aspergillus spp[d]	294	12.31	2.16	12
Curvularia lunata[d]	158	6.61	1.16	9
Chaetomium globosum	12	0.50	0.09	2
Penicillium sp	67	2.80	0.49	6
Tilletia braclayana	16	0.67	0.11	1

Total number of seed borne pathogenic infections recorded on seeds of rice were 2387[b]

Percentage of seed yielding different pathogens was calculated on the basis of 13600 seeds.

Total number of seed samples were 17.

Predominant pathogens constituted at least 5.0% of the total seed borne infection.

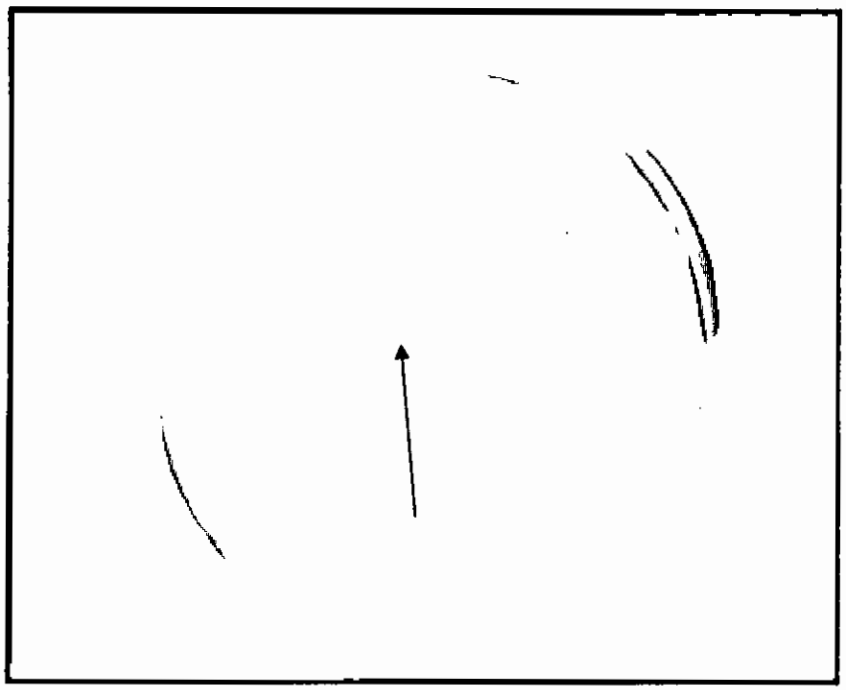


Plate 28. Bacterial ooze in agar plate method

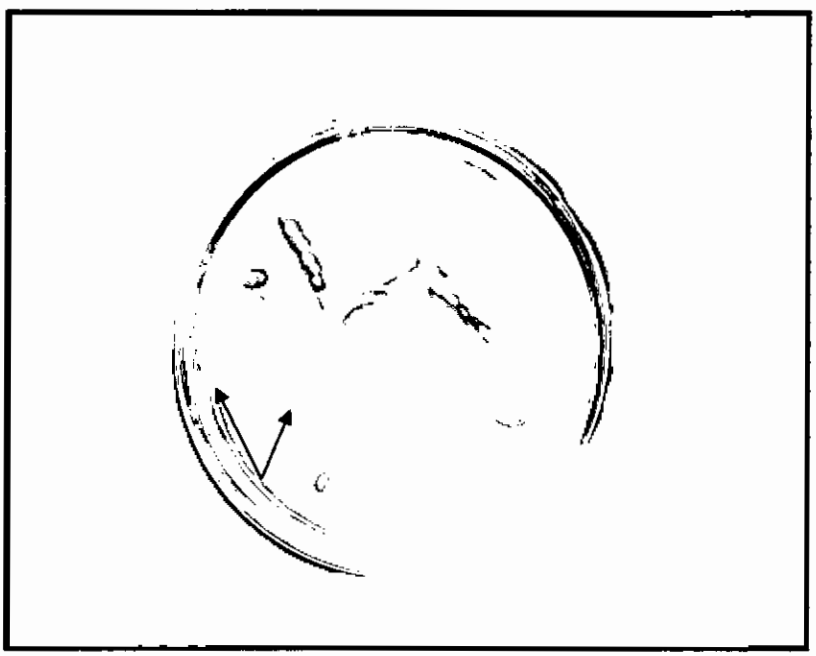


Plate 29. Growth of *Bipolaris oryzae* in agar plate method

Plate 30. Growth of *Curvularia lunata* in agar plate method

Plate 31. Growth of *Fusarium moniliforme* in agar plate method

Chapter 5
Discussion

CHAPTER 5

DISCUSSION

Thirteen imported hybrids, two local hybrids and two local high yielding varieties of rice seed were evaluated to determine the seed health status of imported hybrid rice seed in Bangladesh. A considerable amount of seed borne pathogenic fungi and bacteria were observed by using blotter method, rolled paper towel method and agar plate method.

In total twelve pathogens were associated with the collected seed samples as detected by blotter method and agar plate method. The incidences of different pathogens were found to vary individually and independently among the hybrids and high yielding varieties of rice seeds.

In blotter method, 12 seed borne pathogens were identified. These were *Xanthomonas* spp., *Rhizopus stolonifer, Aspergillus* spp., *Fusarium moniliforme, Phoma sp., Bipolaris oryzae, Curvularia lunata, Penicillium* sp., *Alternaria tenuissima, Nigrospora oryzae, Chaetomium globosum* and *Tilletia barclayana.* It was observed that germination percentage of rice seeds varied significantly from 54.63% to 99.5%. The highest germination was observed in Hira-2 (99.50%) and the lowest germination was recorded in BRRI hybrid dhan-2 (54.63%). Germination percentage of Sonar Bangla-6, BRRI hybrid dhan-1 and BRRI hybrid dhan-2 were below 80% that is lower than the national standard level of rice seed germination. The incidence of *Xanthomonas* spp. ranged from 0.00 to 18.13%. The highest incidence was observed on Sonar Bangla-6 (18.13%) and no incidence was recorded on Hira-1 and Hira-2. Bhutta and Ahmed (1994) reported that maximum seed infection due to *Xanthomonas oryzae* pv. *oryzae* was 11% and 12% in variety IRRI-6 at Lahore and Hydrabad, respectively. The incidence of *Rhizophus stolonifer* ranged from 0.00 to

19.75%. The highest incidence was recorded on BRRI hybrid dhan-1 (19.75%) and no incidence was found in Hira-2 (0.00). The incidence of *Aspergilllus* spp. ranged from 0.00 to 12.00%. The highest incidence was observed on Hira-2 (12.00%) and no incidence was found in Hira-1, Richer-101, Moyna, Tia, Tinpata, Aloron. The incidence of *Fusarium moniliforme* ranged from 0.00 to 9.50%. The highest incidence was observed on ACI-1 (9.50%) and no incidence was on Surma-1, Sonar Bangla-6, Richer-101, Moyna, Tia, Tinpata, Aloron, BRRI hybrid dhan-2, BRRI dhan-29. The incidence of *Phoma* sp. (0.50%) was observed only on Taj-1. The incidence of *Bipolaris oryzae* ranged from 0.00 to 18.00%. The highest incidence was observed on Aloron (18.00%) and there was no incidence was observed on Hira-2, ACI-1, Krishan-2, Sonar Bangla-6, Richer-101, Moyna, Tia, Tinpata, Aloron, BRRI hybrid dhan-2. The incidence of *Curvularia lunata* ranged from 0.00 to 4.25%. The highest incidence was observed on Aloron (4.25%) and no incidence was observed on Hira-1, Hira-2, Surma-1, Taj-1, Sonar Bangla-6, Richer-101, Moyna, BRRI hybrid dhan-1, BRRI dhan-29. The incidence of *Penicillium* sp. ranged from 0.00 to 1.75%. The highest incidence was observed on Modhumoti-2 (1.75%) and no incidence was observed on Hira-1, Hira-2, ACI-1, Surma-1, Taj-1, Sonar Bangla-6, Richer-101,Moyna, Tia and BRRI hybrid dhan-2 . The incidence of *Alternaria tenuissima* ranged from 0.00 to.63%.The highest incidence (0.63%) was observed on Hira-1 and most of the varities showed no incidence (0.00). The incidence of *Nigrospora oryzae* was observed only on Hira-1 (*0.25%*). The incidence of *Chaetomium globosum* (1.25%) was observed only on Modhumoti-2. The incidence of *Tilletia barclayana* (2.75%) was observed only on Richer-101. The present findings were supported previous research reports (Ou, 1972; Fakir and Ahmed, 1974; Hossain and Fakir, 1974 and Sharma *et al.,* 1992). Sharma *et al.,* (1992) detected 10 fungal species of fungi from the rice seeds where *Fusarium moniliforme (Gibberella fujikuroi), Curvularia lunata (Cochliobolus lunata), Aspergillus flavus and Rhizopus* spp. were the most

common. Of all the pathogens *Xanthomonas* spp., *Rhizopus stolonifer* ,*Aspergillus* spp., *Bipolaris oryzae, Fusarium moniliforme* were predominant.These pathogen were designated as predominant, because each of them constituted at least 5.0% of the total seed borne pathogens infection. Mian and Fakir (1989) reported that the most predominant fungi in order of prevalence were *Helminthosporium oryzae, Curvularia lunata, Cladosporium cladosporioides, Aspergillus spp.* and *Trichoconis padwickii.*

. In case of rolled paper towel method seed germination was maximum of 96.38% was recorded on Hira-1 and the lowest of 8.25% germination was recorded on BRRI hybrid dhan-2. Percentage of Non germinated hard seed ranged from 1.63 to 86.50. The maximum percentage of hard seed was recorded on BRRI hybrid dhan-2 (86.50%) and the minimum percentage of hard seed was recorded on ACI-1 (1.63%). Percentage of rotten seed ranged from 12.00 to 0.75. The maximum percentage of rotten seed was recorded on Sonar Bangla-6 (12.00%) and the minimum percentage of rotten seed was recorded on Hira-1 (0.75%). Post-emergence mortality ranged from 0.00 to 4.38%. The maximum percentage of post emergence death recorded on Aloron (4.38%) and no post emergence mortality was recorded on BRRI Hybrid Dhan-2 (0.00%). Vigour index was ranged from 96.36-2329.28. Highest vigour index was in Hira-1(2329.28) and lowest was in BRRI hybrid dhan-2 (96.36). These finding indicate that percent seed germination was decreased due to hard seed and rotten seed. Rotten seed and post emergence mortality of seedling were directly associated with seed borne pathogenic infection.

In agar plate method, 10 seed borne pathogenic infection were identified. These were *Xanthomonas* spp., *Rhizopus stolonifer, Aspergillus flavus, Aspergillus niger, Fusarium moniliforme, Bipolaris oryzae, Curvularia lunata, Penicillium* sp., *Alternaria tenuissima, Nigrospora oryzae* .

The incidence of *Xanthomonas* spp. ranged from 1.63% to 15.13%. The highest incidence of *Xanthomonas* spp. was observed on Tinpata (15.00%) and the lowest incidence was observed on Hira-1(1.63%). The incidence of *Bipolaris oryzae* ranged from 0.00 to 15.13%. The highest incidence was observed on Aloron (15.13%) and no incidence was observed on ACI-1, Taj-1, Krishan-2, Sonar Bangla-6, Richer-101,Moyna, Tia, Tinpata, BRRI hybrid dhan-2.The incidence of *Fusarium moniliforme* ranged from 0.00 to 7.63%. The highest incidence was observed on ACI-1 (7.63%) and no incidence was observed on Surma-1, Sonar Bangla-6, Richer-101, Moyna, Tia, Tinpata, Aloron, BRRI hybrid dhan-2, BRRI dhan-29. The incidence of *Rhizopus stolonifer* ranged from 0.00 to 3.00%.The highest incidence of *Rhizopus stolonifer* was observed on Tia (3.00%) and no incidence was observed on Hira-2, Modhumoti-2. The incidence of *Alternaria tenuissima* ranged from 0.00 to1.63%. The highest incidence was observed on Hira-1(1.63%). The most of the varieties showed no incidence except Modhumoti-2 (1.50%), Krishan-2 (1.25%), Tinpata (1.38%). The incidence of *Nigrospora oryzae* was observed only on Hira-1 (1.38%). The incidence of *Curvularia lunata* ranged from 0.00 to 6.38%. The highest incidence was observed on Aloron (6.38%) no incidence was observed on Hira-1, Hira-2, Surma-1, Taj-1, Richer-101, BRRI Hybrid Dhan-2, BRRI Dhan-29. The incidence of *Penicillium* sp. ranged from 0.00 to 1.88%. The highest incidence was observed on BRRI hybrid dhan-1(1.88%) and no incidence was observed on Hira-1, Hira-2, ACI-1, Surma-1, Taj-1, Richer-101, Sonar Bangla-6, Moyna, Tia, Aloron, BRRI hybrid dhan-2, BRRI Dhan-29. The incidence of *Aspergillus flavus* ranged from 0.00 to 6.50%. The highest incidence was observed on Hira-2 (6.50%) and no incidence was observed on Hira-1, Richer-101 Tia, Tinpata, Aloron. The incidence of *Aspergillus niger* ranged from 0.00 to 1.38%. The highest incidence was observed on Taj-1(1.38%) and no incidence was observed on Hira-1, Hira-2, ACI-1, Sonar

Bangla-6, Richer-101, Moyna, Tia, Tinpata, Aloron, BRRI hybrid dhan-1, BRRI hybrid dhan-2, BRRI dhan-28 and BRRI dhan-2. Of all the pathogens *Xanthomonas* spp., *Bipolaris oryzae, Aspergillus* spp, *Fusarium moniliforme, Rhizopus stolonifer* were predominant.

The fungi and bacteria isoloated in the present studies comprise the genera *Bipolaris, Rhizoctonia, Fusarium, Aspergillus, Penicillium, Alternaria, Nigrospora, Phoma, Chaetomium* and *Xanthomonas* have also been reported in rice seeds by different scientists at home and abroad (Hossain and Fakir, 1974; Ribeiro (1980); Sing and Kang (1987); Basak and Mridha (1988); Fakir *et al.*(1990) Sharma *et at.*(1992), Ilyas and Javid (1995), Ali and Deka (1996). *Bipolaris oryzae, Trichoconis padwickii, Curvularia lunata, Nigrospora oryzae, Alternaria tenuis, Aspergillus* spp. and *Penicillium* spp. were identified by Rahman *et al.* (2000) on BR 11.

Chapter 6

Summary and Conclusion

CHAPTER 6

SUMMARY AND CONCLUSION

The present study was conducted to detect and identify the seed borne pathogens of imported hybrids and varieties of rice in Bangladesh. The experiment was carried out in the seed pathology laboratory and plant disease diagnostic laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 during the period from November 2008 to April 2009. The experiment was carried out according to the rules of International Seed Testing Association (ISTA) with thirteen imported hybrids, two local hybrids and two local high yielding varieties (HYVs). The hybrids and high yielding varieties were Hira-1, Hira-2, ACI-1, Surma-1, Taj-1, Modhumoti-2, Krishan-2, Sonar Bangla-6, Richer-101, Moyna, Tia, Tinpata, Aloron , BRRI hybrid dhan-1, BRRI hybrid dhan-2, BRRI dhan-28, and BRRI dhan-29. In Bangladesh 43 different diseases are known to occur on rice. Among these 27 are seed borne of which 14 are of major importance. In this experiment, twelve pathogens were found and identified by blotter method, rolled paper towel method and agar plate method. These pathogens are *Xanthomonas* spp., *Rhizopus stolonifer, Aspergillus* spp. (*Aspergillus flavus, Aspergillus niger*), *Bipolaris oryzae, Fusarium moniliforme, Curvularia lunata, Alternaria tenuissima, Nigrospora sp. Chaetomium globosum ,Tilletia braclayana* and *Phoma* sp. The incidence of different seed borne pathogens was found to vary individually and independently among the hybrids and high yielding varieties of rice seeds.

In blotter method, highest incidence of *Xanthomonas* spp., *Rhizopus stolonifer, Aspergillus* spp., *Fusarium moniliforme*, *Bipolaris oryzae* , *Penicilium* sp., *Curvularia lunata, Alternaria tenuissima* was observed on Sonar Bangla-6, BRRI hybrid dhan-2, Hira-2, ACI-1, Aloron, Modhumoti-2,

Aloron, Hira-1, respectively. Some pathogens were recorded only on one variety. Such as *Phoma* sp. on Taj-1, *Nigrospora* sp. on Hira-1, *Chaetomium globosum* on Modhumoti-2 and *Tilletia barclayana* on Richer-101 were recorded. Of all the pathogens *Xanthomonas* spp., *Rhizopus stolonifer, Aspergillus* sp., *Bipolaris oryzae,*
Fusarium moniliforme were predominant.

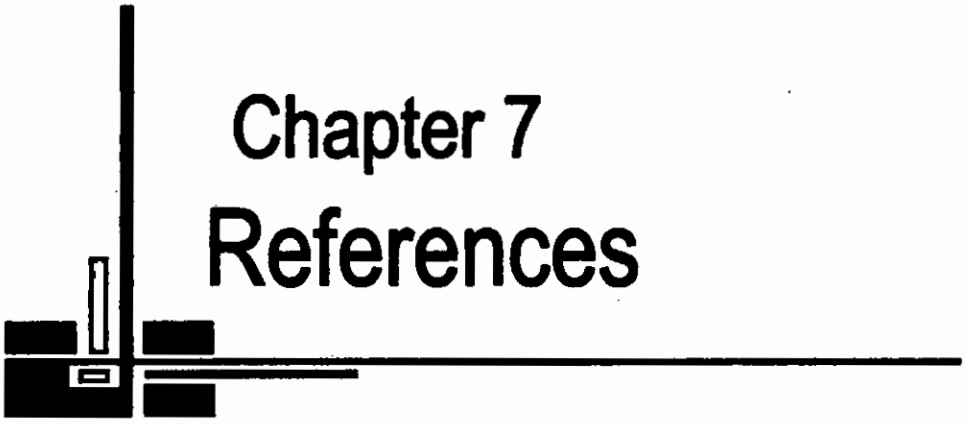
In rolled paper towel method, the highest percentage of non-germinated seed (hard and rotten seed) was recorded on Moyna and Sonar Bangla-6 respectively. The highest post-emergence mortality was noticed on Aloron. The vigour index was higher in Hira-1 with lower pathogenic infection compared to other varieties. Percent seed germination was decreased due to hard seed and rotten seed. Post emergence mortality of seedlings and non germinated rotten seeds were directly associated with the seed borne pathogenic infection.

In agar plate method, the highest incidence of *Xanthomonas* spp. was noticed on Tinpata where as *Bipolaris oryzae* on Aloron, *Fusarium moniliforme* on ACI-1, *Rhizopus stolonifer* on Tia, *Alternaria tenuissima* on Hira-1, *Curvularia lunata* on Aloron, *Penicilium* sp and *Aspergillus flavus* on BRRI hybrid dhan-1, *Aspergillus niger* on Taj-1 were observed. *Nigrospora* sp. was recorded only on Hira-1. Of all the pathogens *Xanthomonas* spp., *Bipolaris oryzae, Aspergillus* sp, *,Fusarium moniliforme Rhizopus stolonifer* were predominant.

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 pathogen. So pathogen free seed is the vital input in agriculture. Rice seed play an important role to carry pathogen from abroad and within the country. Now Bangladesh government give emphasis to cultivate

hybrid rice to meet the food demand of the country. And most of the hybrid rice seeds are imported. The imported hybrid Varieties are treated with seed treating chemicals for maintaining quarantine regulations. But from the present study it was revealed that a lot of seed borne pathogens were associated with those seeds. It was also noticed that a particular pathogen was observed in a particular varity. Such as *Phoma sp., Tilletia baraclyana, Nigrospora sp. Xanthomonas* spp., *Bipolars oryzae, Aspergillus* spp., *Fusarium moniliforme, Rhizopus stolonifer* are identified predominant seed borne pathogens found from the imported hybrid rice seeds.

Considering the overall findings, it was revealed that the seed health status of imported hybrid rice seeds was not at satisfactory level. However, further study will need to be carried out for consicutive years including all of the imported and local rice varieties in different Agro Ecological Zones (AEZs) and different cropping season of the country.



Chapter 7
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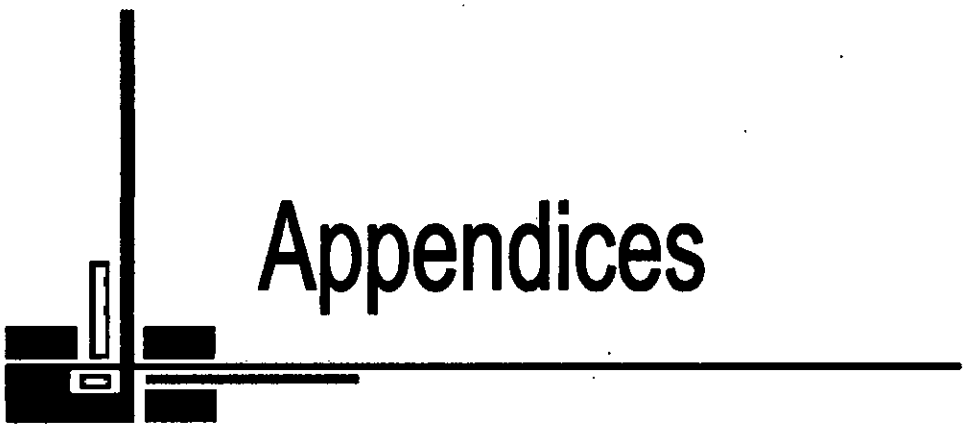

Appendices

Appendix I. Analysis of variance of the data on percent seed germination on hybrids and high yielding varieties of rice seed by blotter method and rolled paper towel method

Source of variation	Degrees of freedom	Mean square value	
		% germination	
		Blotter method	Paper Towel Method
Replication	3	16.48	13.426
Variety	16	369.18*	248.53*
Error	48	3.426	5.419

Appendix I I. Analysis of variance of the data on percent Non germinated seed (hard seed and rotten seed) and post-emergence mortality of hybrids and high yielding varieties of rice by rolled paper towel method

Source of variation	Degrees of freedom	Mean square value		
		Non germinated seed		Post-emergence mortality
		Hard seed	Rotten seed	
Replication	3	57.71	0.83	3.18
Variety	16	249.09*	41.51*	5.80**
Error	48	11.884	4.138	0.259

Appendix III. Analysis of variance of the data on percent incidence of pathogen on hybrids and high yielding varieties of rice by blotter method

Source of variation	Degrees of freedom	Mean square value											
		%Pathogen incidence											
		Xanthomons spp.	*Rhizopus stolonifer*	*Aspergillus* Spp.	*Fusarium moniliforme*	*Phoma* sp.	*Bipolaris oryzae*	*Curvularia lunata*	*Penicillium* Sp.	*Alternaria tenuissima*	*Nigrospora* Sp.	*Chaetomium globosum*	*Tilletia barclayana*
Replication	3	6.121	2.34	0.188	0.995	0.145	0.008	0.726	0.164	1.922	0.029	0.031	0.016
Variety	16	67.54*	58.61*	16.43*	2.49**	2.61**	0.48**	8.67*	2.93**	10.62*	1.73**	1.08**	0.246**
Error	48	4.612	1.923	0.866	0.908	0.159	0.014	0.588	0.128	1.888	0.224	0.096	0.028

Appendix IV. Analysis of variance of the data on percent incidence of pathogen on hybrids and high yielding varieties of rice seed by agar plate method

Source of variation	Degrees of freedom	Mean square value									
		%Pathogen incidence									
		Xanthomons spp.	*Bipolaris oryzae*	*Fusarium moniliforme*	*Rhizopus stolonifer*	*Alternaria tenuissima*	*Nigrospora* sp	*Curvularia lunata*	*Penicilium* sp.	*Aspergillus flavus*	*Aspergillus niger*
Replication	3	7.122	1.324	0.167	1.795	0.142	0.004	0.925	0.063	1.922	0.029
Variety	16	67.582*	69.321*	19.258*	2.649**	1.600**	0.445**	10.673*	1.963**	11.631*	0.843**
Error	48	3.841	0.962	0.974	0.916	0.056	0.004	0.519	0.117	1.888	0.024

Appendix V. Analysis of variance of the data on root length, shoot length and vigour index of hybrids and High yieldingvarieties of rice seed by rolled paper towel method

Source of variation	Degrees of freedom	Mean square value		
		Root length (cm)	Shoot length (cm)	Vigour index
Replication	3	0.297	0.026	4916.687
Variety	16	29.38**	14.99**	17223.853*
Error	48	0.523	0.154	560.573