SEED BORNE FUNGI AND SEEDLING VIGOUR OF WHEAT SEEDS COLLECTED FROM SOUTH WESTERN REGION OF BANGLADESH

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CERTIFICATE

This is to certify that the thesis entitled, **"SEED BORNE FUNGI AND SEEDLING VIGOUR OF WHEAT SEEDS COLLECTED FROM SOUTH WESTERN REGION OF BANGLADESH''** submitted to the Department of Plant Pathology, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in the partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M. S.) IN PLANT PATHOLOGY,** embodies the result of a piece of bonafide research work carried out by **NISHAT TASNIM SIDDIKA Registration No. 14-06345** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma in anywhere in the world.

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

Dated: 1.12.2016
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DEDICATED TO

MY

BELOVED PARENTS

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ABSTRACT

Twenty wheat seed samples collected from south western region of Bangladesh were tested for seed borne fungi, germination and seedling vigor, following different methods namely blotter method, rolled paper towel method and deep freezing blotter method. Water agar test tube seedling symptom test were also done. Seed borne fungi viz *Bipolaris sorokiniana* , *Aspergillus flavus, Aspergillus niger, Alternaria tenuis, Fusarium moniliforme, Penicillium* spp., *Curvularia lunata* and *Pyricularia grisea* were isolated from three wheat varieties Shatabdi, Prodip and Sonalika. Incidence of seed borne fungi were recorded and compared to different seed health testing methods. Incidence of different seed yielding fungi ranged from 0.33 to 26.33% and 0.67 to 30.00% in blotter paper and deep freezing blotter method, respectively. Seed germination percent ranged from 2.73 to 66.40% and 60 to 81% for rolled paper towel method and seedling symptom test respectively. Percent normal seedling (46.67%) was higher in Sonalika variety and percent abnormal seedling (50%) was higher in Prodip variety. Seedling vigor ranged from 480.0 to 1964 for Shotabdi, 1450 to 1844 for Prodip and 1130 to 1796 for Sonalika variety .

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LIST OF SYMBOLS AND ABBREVIATIONS

No.	=	Number
%	=	Percentage
et al.	=	And others
^{o}C	=	Degree Celsius
@	=	At the rate of
etc.	=	Etcetra
J.	=	Journal
Viz.	=	Namely
Cm	=	Centimeter
Cfu	=	Colony forming unit
df.	=	Degrees of freedom
&	=	And
ppm	=	Parts per million
Kg	=	Kilogram
g	=	Gram
ml	=	Milliliter

LIST OF SYMBOLS AND ABBREVIATIONS (Cont'd)

hr	=	Hour (s)
i.e.	=	That is
T	=	Treatment
cv.	=	Cultivar (s)
var.	=	Variety
mm	=	Millimeter
µl	=	Microliter
µm	=	Micrometer
SAU	=	Sher-e-Bangla Agricultural University
BBS	=	Bangladesh Bureau of Statistics
USA	=	United States of America
ANOVA	=	Analysis of Variances
LSD	=	Least Significant Difference
CV%	=	Percentages of Co-efficient of Variance

CHAPTER 1

INTRODUCTION

Wheat (*Triticum aestivum L*.) is one of the major widely cultivated cereal food crop in the world. It is one of the most important staple foods of man and is grown in almost all the temperate and sub-tropical regions of the world . Wheat is one of the important cereal crops and major food grain in Bangladesh (Mamun and Shahjahan, 2011). It is considered as the second staple cereal crop next to rice in Bangladesh (Podder *et al*., 2012). Wheat grains are highly nutritive and rich in energy, carbohydrates, dietary fibre, fat, protein, thiamine, riboflavin, niacin, pantothenic acid, vitamin B6, folate, calcium, iron, magnesium, phosphorus, potassium, zinc and manganese (USDA, 2014). Due to the high nutritive value, wheat grains are eaten in various forms across cultures and continents (Acharya *et al*., 2011).

In Bangladesh, the area as well as yield of wheat has been increased manifold due to the initiation of high yielding variety (HYV) of wheat expansion program in 1974 (Ahmed and Meisner, 1996). In Bangladesh, wheat covered almost 411706.8 hectares of land with a total production of 1254778 metric tons (BBS, 2012). Though the area, production and yield of wheat have been increasing during the last decades, the average wheat yield in Bangladesh is comparatively too low (3.01mt/ha) in comparison to major wheat growing countries of the world like France

(7.6mt/ha), Japan (4.10mt/ha), China (4.98mt/ha), USA (3.12mt/ha) and India (4.20mt/ha) (Podder *et al*., 2012; FAO, 2012).

There are many constraints responsible for lower yield of wheat in Bangladesh. Among those use of unhealthy or diseased seeds is one of the major constraints (Panna *et al.,* 2009). Primary source of the infection for some of the diseases are the grains itself (Ali and Fakir, 1982). Seed borne pathogen may cause seed abortion, seed rot, seed necrosis or reduction in germination as well as seedling damage by systemic or local infection resulting the development of disease at later stages of plant growth (Khanzada *et al*., 2002). Wheat suffers from as many as 120 different diseases in different parts of the world and out of them 42 is seed borne and 35 are caused by fungi (Richardson, 1979).

Seed-borne fungi are the most important biotic constrains in seed production worldwide. They are responsible for both pre and post-emergence death of grains, affect seedling vigor, and thus cause some reduction in germination and also variation in plant morphology (Van Du *et al.,* 2001; Rajput et al., 2005; Niaz and Dawar, 2009). The seedborne pathogens may result in germination failure, discoloration and shriveling of grains, occurrence of plant diseases, distribution of pathogen to new areas, introduction of new strains or physiologic races of the pathogen along with new germplasm from one country to other countries and toxin production in infected seed (Agarwal and Gaur, Undated). Fungi and all other types of pathogens that attack plants and cause a very serious economic impact on agricultural production due to their ability to induce

diseases of cultivated crops that result in important yield losses (Paplomatas, 2006) .

Quality features of wheat seeds, such as seed germination, moisture content, seed discolouration and seed-borne fungal prevalence have long been known to be influenced by various factors.

The major mycotoxigenic fungi are *Aspergillus* spp., *Fusarium* spp., and *Penicillium* spp. in cereal. The type and severity of seed abnormality may be dependent on the type and pathogenic potential of the associated fungi as well as the prevailing weather conditions.

The harmful effects of such fungal invasion are glume or grain discolouration. Other abnormalities such as deformation and damage in seeds are of major constraint in crop production in most of the developing countries. Seed abnormality due to the influence of seedborne fungi is very common and often accounts for a large percentage of crop losses. Various experiments have been conducted in the last three decades to find out the effective means of isolating and controlling seed fungi form different crops.

The present research work was therefore designed with the following specific objectives:

1. To detect the fungi associated with wheat seeds collected from south western region of Bangladesh.

2. To record the germination and seedling vigor of wheat seeds collected from south western region of Bangladesh.

3. To analyze the variation of presence of seed borne fungi in terms of variety and seed health testing method.

CHAPTER 2

REVIEW OF LITERATURE

2.1. Seed-borne fungi associated with wheat seeds

Abdullah and Atroshi (2015) recorded eight fungal species on wheat grains for the first time in Iraq. These included *Aithrinium phaeospermum, Bipolaris sorokiniana, B. spicifera, Chaetomium datum, Emericella rugulosa, Eurotium herbariorum Nigrospora state* of *Khuskia oiyzae* and *Ulodadium alternariae*.

Awad and Baka (2014) conducted an experiment with grain samples of 14 Egyptian wheat cultivars for testing of seed-borne fungi using deep freezing method. Five seed-borne fungi viz. *Aspergillus flavus, A. niger, Curvularia lunata, Fusarium moniliforme* and *Penicillium chrysogenum* were isolated from different wheat cultivars. *A. flavus, A. niger* and *F. moniliforme* were the most prevalent fungal species. Their incidence ranged from 21.0-53.5%, 16.0-37.5%, and 12.0-31.0%, respectively. The antifungal potential of water extracts from aerial parts of five wild medicinal plants (*Asclepias sinaica, Farsetia aegyptia, Hypericum sinaicum, Phagnalon sinaicum* and *Salvia aegyptiaca*) was collected from the Sinai Peninsula, Egypt. All the aqueous plant extracts significantly reduced the incidence of the tested seed-borne fungi. But the extract of

Asclepias sinaica exhibited the most antifungal activity on tested fungi at all concentrations used when compared with other plant extracts. Treating grains with plant extract of *A. sinaica* (10%) enhanced the percentage of grain germination of all cultivars in both laboratory and pot experiments. Maximum root and shoot length of seedlings was recorded in Bani Suef 4 during fungal infestation or treatment by plant extract. For one hour before sowing or storage, the aqueous extract of *A. sinaica* can be used to treat wheat grains, to reduce the fungal incidence. Aqueous extracts of the aerial parts of selected medicinal plants, particularly *A. sinaica*, are promising for protecting Egyptian wheat grain cultivars against major seed-borne fungi.

Pathak and Zaidi (2014) made a study in order to evaluate the infection and identication of different fungal genera associated with storage wheat varieties. Total loss due to seed-borne diseases is up to an extent of 30-75%. Wheat varieties grown during the months of October to March 2011, collected from Quarsi agricultural farm, Aligarh were screened by using Blotter method, Agar plate method and Deep freeze method as recommended by ISTA. Out of the five varieties tested, PBW343 was found to be most susceptible and was associated with more fungal flora than the other varieties. Seed mycoflora of abnormal seeds was also studied. Maximum incidence of fungi was observed in case of discolored seeds followed by shrunken seeds and cracked seeds. In all the three detection methods, a total no of 11 genera and 20 species of fungi were isolated and identified.

Bishaw *et al*. (2013) made a study in order to assess the seed health quality of wheat (*Triticum aestivum*) seed samples collected from formal and informal sector in Ehiopia and Syria. In Ethiopia, several seed borne fungi viz. *Cochliobolus sativum, Fusarium avenaceum, F. graminearum, F. nivale, F. poae* and *Septoria nodorum. C. sativum* was predominant with 84% of samples infected (frequency) and 1.85% mean infection level (rate) followed by *F. graminearum* with 74% and 1.54%, respectively. In Syria, 68% and 14%, respectively, of wheat samples were infected with common bunt (*Tillia* spp.) and loose smut (*Ustilago tritici).*

El-wakil (2013) conducted an experiment with nine samples of wheat grains cv. Sakha 69, for screening of the associated fungi and 15 fungal species belonging to eight genera were isolated from wheat seeds. The isolated fungi were *Aspergillus flavus, Aspergillus niger, Aspergillus ochraceous, Aspergillus parasiticus, Alternaria alternata, Stemphylium* spp., *Cladosporium spp., Drechslera spp. Fusarium solani, Fusarium moniliforme, Fusarium semitectum, Fusarium nivale, Fusarium oxysporum, Penicillium* spp. and *Trichoderma* spp. The genus *Aspergillus* gave the highest percentage of seed colonization of the isolated fungi followed by *Fusarium* spp.

Hussain *et al.* (2013) reported that seed borne mycoflora associated with ten comercial varieties of wheat viz. Blue silver, Faisalabad 85, Manthar-3, Pak 81, Parwaz 94, Pirsabaq 2005, Punjnad-1, Sariab-92, Sh-2002 and Wafaq-2001 was investigated through standard blotter paper and agar

plate method by using Mann-Whitney U test. At least eleven fungal genera were recovered from seeds. The most frequently isolated fungi were *Bipolaris sorokiniana* (11.125%), *Aspergillus flavus* (9.82%), *Alternaria alternata* (7.15%) and *Aspergillus niger* (6.22%). It is apparent from the present investigation that all commercial wheat varieties tested were contaminated by fungi. The rolled paper method was used to find out the effect of seed borne fungi on seed germination. Seeds of Pak 81, Wafaq-2001 and Blue silver were germinated in high proportion with variable number of normal and abnormal seedlings than the seeds of other varieties tested. The fungi associated with seeds of wheat cause dire diseases in wheat reducing the germination capacity.

Pathak and Zaidi (2013a) conducted an experiment to ascertain the fungal species and their effect on germination associated with wheat seeds. Seeds of three varieties WH896, PBW-373 and HD264 of wheat (*Triticum aestivum*) were collected from Quarsi Agriculture Farm, Aligarh. These three seed samples of wheat showing different forms of discouloration and abnormalities were screened for associated fungi. Microscopic examination of wheat seeds reveals that seeds of all the varieties of wheat possess injuries to varying extent. Detailed examination of the seeds has shown that the seeds can be classified on the basis of extent of injury in the three categories viz. seeds having minor cracks, cracks without exposed embryo and cracks with exposed embryo.

Pathak and Zaidi (2013b) studied seed mycoflora associated with wheat on different media with a particular reference to Blotter and potato dextrose agar (PDA) procedures of ISTA. Seed-borne fungi, viz. *Fusarium moniliforme, Rhizopus* spp*., Mucor* spp., *Alternaria alternata*, *Aspergillus niger, Aspergillus flavus, Curvularia lunata, Drechslera* spp, *Alternaria* spp. and *Penicillium* spp. were isolated from the variety HD264. Blotter method was found to be the best media for the isolation of mycoflora whether borne externally or internally.

Zrari (2013) isolated and identified ten seed borne fungi (*Alternaria* spp., *Aspergillus* spp., *Aureobasidium* spp., *Cladosporium* spp., *Dreschslera* spp., *Penicillium* spp., *Rhizoctonia* spp., *Stemphylium* spp., *Mucor* spp. and *Rhizopus* spp.) from two wheat varieties. The highest frequency of seed borne fungi was observed on wheat cultivar site Mol14. The mean and standard deviation of *Alternaria* spp. was (5.5± 1.69) while the lowest frequency fungal isolated was *Dreschslera* spp. and *Rhizopus* spp. Their mean and standard deviation was (0.1± 0.64).

Hajihasani *et al.* (2012) made a study in Markazi province in the central of Iran with 53 seed samples collected from harvested seed loads of irrigated wheat fields. A total of 15 fungal species including *Tilletia laevis, Tilletia tritici, Ustilago tritici, Fusarium graminearum, Fusarium culmorum, Microdochium nivale, Bipolaris sorokiniana, Alternaria alternata, Curvularia* spp., *Aspergillus niger, Aspergillus candidus, Aspergillus flavus, Penicillium* spp., *Mucor* spp. and *Rhizopus* spp. were identified in three wheat cultivars of Backcross Roshan, Alvand and C-78-14.The average of infection level in tested samples to both *T. laevis* and *T. tritici* was

estimated as much as 7.1% in the province and the minimum and maximum infection levels were found in Lilian (Khomein) and Jirya regions (Arak), respectively. The average of infection rate by *U. tritici* in seed samples was 1.3% while it was as much as 17.4% for both *F. culmorum* and *B. sorokiniana* in the province. The frequency of *A. niger* and *Penicillium* spp. was predominant with an infection range of 37.8 and 29.1%, respectively.

Acharya *et al.* (2012) reported that in recent years, spot blotch disease, caused by *Bipolaris sorokiniana* (Sacc.) Shoem. syn. *Drechslera sorokiniana* (Sacc.) Subrm and Jain (syn. *Helminthosporium sativum*, teleomorph (*Cochliobolus sativus*) have emerged as serious concern for cultivation in warmer and humid regions of the world. During past two decades, substantial economic loss in wheat production has occurred due to the severity of spot blotch, affecting the livelihood of millions of small-scale farmers. Besides spot blotch, this fungus is also the causal agent of other diseases like common root rot, foot rot, seedling blight and seed rot of wheat. The greatest yield losses occur when the flag leaf and the leaf below the flag leaf become infected before the emergence of head.

Mobasser *et al*. (2012) made a study in order to evaluate the informal wheat seed contamination with seed-borne diseases in two most important provinces (East Azarbaijan and Khorasan Razavi) for wheat production in cold region of Iran in 2008-2009 crop seasons. Seed health (head blight, common bunt and loose smut disease) tests were carried

out on seed samples according to ISTA rules. *Fusarium graminearum* was identified as the main disease in provinces. Washing test for *T. caries* showed significant difference between and among provinces and towns with respect to *T. leavis* infection.

Sultana and Rashid (2012$_a$) conducted an experiment in vitro to determine the effect of wheat seed categories such as healthy looking, blackpointed and shriveled as affected by *Bipolaris sorokiniana* on the germination of wheat seeds. The work was done in the laboratory of Seed Pathology Centre, Bangladesh Agricultural University, Mymensingh during 2010-11. The highest prevalence (65%) of *B. sorokiniana* was recorded in shriveled seeds, (42%) was recorded in black pointed seeds and (30%) was associated with healthy looking seeds.

Sultana and Rashid (2012$_b$) conducted an experiment in vitro to determine the planting value of wheat seeds as affected by *Bipolaris sorokiniana*. The wheat seed samples were tested in blotter method and impairment caused by *B. sorokiniana* was recorded after sprouting of the seeds. During the germination of wheat seeds the pathogen transmitted from seed to plant and caused germination failure, coleoptile infection and root infection. Regression between prevalence of the pathogen and germination failure along with the coleoptiles and coleorhizae infection indicated the increasing trend of deteriorating planting value of the seed with the increasing rate of transmission of the pathogen from seed to germinating seed and seedlings.

Jabber (2011) conducted an experiment in vitro to detect the fungi associated with wheat seeds collected from ten unions of sadar upazila of Thakurgoan district. The health status of 20 seed samples was determined and total six fungi belonging to five genera were identified. The recorded fungi were *Bipolaris sorokiniana, Alternaria tenuis, Fusrium* spp., *Aspergillus flavus, Aspergillus niger* and *Penncillium* spp. Prevalence of total as well as individual seed borne fungal infection were found significantly in respect to wheat varieties and sources of seed collection. Seed samples collected from Jagonathpur and Gorea unions of sadar upazila showed highest percentage of seed borne infection compared to the samples collected from other unions for both varieties. Seed germination also varied significantly depending on the varieties and the seed sources.

Monsura and Rashid (2011) reported that the newly released 22 wheat varieties collected from WRC were categorized into large healthy, small healthy, black pointed, and shriveled and tested by blotter method. Prevalence of different fungi associated with the seed was recorded as *Alternaria tenius*, *Aspergillus* sp., *Bipolaris sorokiniana, Curvularia* sp., *Epicoccum* sp. and *Fusarium* sp.. The highest prevalence of *Bipolaris sorokiniana* (20%) on Pavon.

Singh *et al.* (2011) made a study to enumerate the fungal species and their effect on germination associated with wheat seeds. Seeds of two

cultivars viz. Kundan and HUW-234 of wheat (*Triticum aestivum L*.) were collected after harvesting from agriculture farm, Banaras Hindu University, Varanasi. These seeds were treated with potassium nitrate and examine for seed mycoflora by agar plate method and blotter method. Total sixteen fungal species were isolated from test cultivars by the standard techniques. Fungi isolated and identified were *Alternaria alternata, Alternaria solani, Aspergillus candidus, Aspergillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus terreus, Curvularia lunata, Fusarium roseum, Fusarium semitectum, Penicillium citrinum, Penicillium rubrum, Rhizopus stolonifer, Trichoderma harzianum*. During isolation, the blotter method yielded the higher number of fungi as compared to agar plate method.

Laila *et al*. (2010) conducted to study the reaction of some wheat varieties to *Bipolaris sorokiniana* in vitro. The wheat seeds of varieties Sonalika, Kanchan, Barkat, Shatabdi, Aghrani, Pavon-76, Akbar, Gourab, Sourav and Protiva were collected from Bangladesh Agricultural Research Institute (BARI), Gazipur. In blotter test maximum germination (92%) was recorded in the variety Shatabdi, Sourab and Agrani, and minimum (72%) was recorded in Sonalika. Maximum (37%) incidence of seed borne Bipolaris sorokiniana was recorded in Sonalika and minimum (12%) in Shatabdi.

Iftekher *et al*. (2009) reported that *Bipolaris sorokiniana* (Sacc.) Shoemaker (teleomorph Cochliobolus sativus) was the causal agent of

common root rot, leaf spot and seedling blight, head blight of wheat and barley and black point of grains. It caused significant yield losses in South Asian countries and considered as a serious foliar disease constraints in warmer growing areas. Numerous plant species other than wheat and barley are identified as the host of *Bipolaris sorokiniana* world wide.

Chowdhury (2008) reported that the reduction of germination was found in respect to the severity of black point infection. Argentina reported its first blast infections and associated losses in a summer seeded wheat experimental crop in the north-eastern state of Chaco in 2007/08 (Alberione et al. 2008).

Karwasra *et al*. (2007) reported that the effect of black point disease caused by *Drecslera sorokiniana* or *Alternaria tenuis* on the seed vigor-related parameters (1000-grain weight, germination, and shoot and root growth) of bold-seeded (Sonalika) wheat cultivars were tested and the result found that the germination percentage decreased markedly with the increase in the severity of infection. Shoot and root growth also decreased as the level of infection increased.

Duan *et al*. (2007) observed that total 1465 wheat accessions were tested for detection of seed borne fungi. By blotter test, 17 genera of fungi, including more than 30 species, were detected in 712 wheat accessions. *Alternaria* was the most frequently detected in wheat seeds, followed *by Rhizopus, Penicillium, Aspergillus, Bipolaris, Cladosporium, Gonatobotrys,*

Chaetomium and others. Totally 19 genera of fungi were detected in wheat seed samples and some seed borne fungi were saprophytic and others were biotrophic which could cause seed borne diseases in the field.

Zishan et al. (2007) reported that seed samples of eight wheat cultivars were tested for isolation of pathogens associated with black point at the Department of Plant Pathology, NWFP Agricultural University, Peshawar. *Alternaria alternata* was the predominant pathogen associated with the diseased seeds with high incidence while *Curvularia lunata* was detected with low frequency from two cultivars in both seasons.

Javid and Anjum (2006) conducted a study to identify the presence fungi associated with stored seeds of economically important crops of Pakistan such as wheat (*Triticum aestivum*), Rice (*Oryza sativa*), maize (*Zea mays*), chickpea (*Cicer arietinum*), sunflower (*Helianthus annuas*), soyabean (*Glycine max*), mungbean (*Vigna radiata*), pea (*Pisum sativum*), ground nut (*Arachis hypogea*), tomato (*Lycopersicon esculentum*) and sorhum (Sorghum bicolor) in Pakistan and their control measures. Species of the pathogenic fungal genera viz. *Alternaria, Fusarium, Graminearum, Helminthosporium, Dreschlera, Curvularia, Cercospora, Macrophomina* and *Clasporium* and storage fungi including *Aspergillus* and *Penicillium* are generally associated with of these crop.

Morejon *et al*. (2006) reported that diseases caused in wheat by *Helminthosporium* spp. have led to considerable yield and production losses. Different species in this genus are associated with wheat seeds. The current study was undertaken to identify the most frequent fungus species that normally infects wheat seeds and compared them with *B. sorokiniana*. The fungus *Bipolaris bicolor*, isolated from wheat seeds cultivar IAPAR, was identified by taxonomic methods and compared with the fungus *B*. *sorokiniana*, in relation to growth characteristics on the seeds, as well as to growth characteristics in PDA and morphology of the structures. Type of colony observed on the seeds is important for the differentiation between the fungus species*. B. sorokiniana* presented black colonies, which were well-adherent to the seeds, whereas *B. bicolor* presented grayish, aerial, cotton like colonies. The size of the conidia also differed in length and width, and B. bicolor presented the smallest dimensions. In relation to septa*, B. bicolor* conidia presented deep ones, with dark color bases, but seldom presented dark apex. *B. sorokiniana* presented homogenous color.

Enikuomehin (2005) investigated the health of wheat seeds produced under rain- fed conditions in South Western Nigeria . There were more abnormal (1.0 to 79.7%) than normal (10.7 to 28.7%) seeds. Forms of seed abnormality observed include wrinkled seeds (64.2 to 79.7%), entirely discoloured seeds (1.0 to 12.5%), seeds with discoloured. embryo (germ) (1.2 to l.5%) and brush (0.25 to 1.25%) ends*. Fusarium graminearum* and *Helminthosporium sativum* were associated with all seeds, but at higher levels in abnormal (*Fusarium graminearum*, 0.5 w

78.5%; *H. sativum*, 2.5 to 86.0%) than normal seeds (*Fusarium graminearum*, 2.25%; and *H. sativum*, 0.75%). Viability of abnormal seeds was 1.50 to 32.0% whick was much lower than the 88.0% germination of normal seeds.

Khokon *et al.* (2005) reported that germination of wheat seeds was negatively correlated to seed infection and significantly affected by seed infection by *Bipolaris* sp.

Kolawole *et al*. (2005) carried out a study to determine the post-harvest fungi of wheat circulating in Lagos State, Nigeria. A total of 400 wheat samples from eight major market of wheat in Lagos State, were collected randomly from the seller of this agricultural produce. Results from this study revealed that *Penicillium* spp. 90(22.5%) as the most predominant, followed by *Aspergillus flavus* 70(17.5%) while *Trichoderma* spp. was the least isolated fungal flora of wheat with a prevalent rate of 14(3.5%). Also, all the isolated mycoflora of wheat were found to be pathogenic, but to varying degree of virulence, as shown by their percentage rate of infection. The order of their pathogenicity are as follows*: Aspergillus niger, Aspergillus flavus, Aspergillus fumigatus* and *Penicillium* spp. 50(100%) >*Rhizopus* spp. 50(90%) >*Fusarium solani* 50(80%)>*Alternaria* spp. and *Trichoderma* spp. 50(70%). It can, therefore, be concluded that, wheat circulating in Lagos State, Nigeria are variously contaminated with different xerophilic molds.

Rajput *et al*. (2005) made a study in Sindh wheat growing areas where one hundred twenty wheat seed samples were collected and tested for fungal seed- borne pathogens by using the standard blotter method. Five seed borne fungi viz. *Alternaria tenuis*, *Aspergillus niger*, *Fusarium moniliforme*, *Curvuluria lunata* and *Stemphylium herhurum* were isolated from 12 wheat varieties viz. Mehran, T.J-83, Soghat, Sarsabz, Anmol, Johar, C-591, Sindh-81, Pak-70, Mexipak-65, H- 68 and Faisalabad-85, respectively. *Alternaria tenuis* was predominance with an infection range from 22.5-47.5%. Maximum seed germination was observed in Anmol and minimum in Pak-70. Maximum root and shoot length of seedlings was recorded in Anmol and Sarsabz followed by H-68 and minimum in Pak-70, Mehran-89, Soghat and Johar.

Wang *et al*. (2003) reported that kernel discolorations of wheat *(Triticum* spp.), as observed in black point, smudge, penetrated smudge, red smudge, and *Fusarium*- damaged kernels, were important downgrading factors in western Canada. Black point, which was often associated with infections by fungi such as *Alternaria alternata*, *Cochliobolus sativus* and *Pyrenophora triticirepentis,* was characterized by a distinct dark brown or black discoloration of the whole germ and surrounding area. This discoloration was referred to as 'smudge' when more than one-half of the kernel is discolored or the discoloration extends into the increase, and as 'penetrated smudge' when the discoloration penetrates and extends throughout the endosperm.

Malaker and Mian (2002) reported that the incidence of *Bipolaris sorokiniana* increased with increasing severity of black point infection. Reduction in germination, seedling emergence, plant stand, root and shoot growth, vigour index and grain yield were directly related with the severity of black point.

Malaker *et al*. (2002) conducted a study to determine the effect of different grades of black point infection on seed quality and yield of wheat cv. Kanchan. Freshly harvested wheat seeds were categorized into six grades, viz., seeds free from any discoloration i.e. apparently healthy, seeds with only tip of the embryo brown to blackish, seeds with discoloration covering the whole embryo, seeds with embryo and one-fourth of the grain discoloured, seeds with embryo and half of the grain discoloured and seeds with embryo and more than half of the grain discoloured and shrivelled. Seed health test on blotters showed that the major fungi associated with black point affected seeds, in order of prevalence, were: *Bipoluris sorokiniana (Cochliobolus sativus), Alternaria alternata, Curvularia lunata* (*Cochliobolus lunatus*) and *Fusarium* spp. The incidence of *Bipolaris sorokiniana* increased with increasing severity of black point infection. Reduction in germination, seedling emergence, plant stand, root and shoot growth, vigour index and grain yield were directly related with the severity of black point.

Ammara *et al*. (2001) reported that fungal and bacterial incidence and frequency of occurrence on wheat seeds, as well as their impact on seed

germination were investigated. The main pathogen incidence of seed borne pathogens was 66.16% on seeds of nine wheat cultivars, whereas fungal, bacterial and black point incidence was 47.12, 12.28 and 6.76%, respectively. The frequency of occurrence of seed borne pathogens and their impact on seed germination varied from cultivar to cultivar. Field fungal pathogens including *Alternaria tenuis (Alternaria alternata), Curvularia lunata (Cochliobolus lunatu), Fusarium graminearum (Gibberella zeae), Helminthosporium sativum (Cochliobolus sativus)* and *Phoma* sp., two storage fungi included *Aspergillus* and *Penicillium* sp. and one bacterium namely *Xanthomonas campestris* pv. translucens were identified from seeds as well as abnormal seedlings. The seed borne pathogens reduced seed germination.

Giri *et al*. (2001) reported that seed samples of different wheat varieties were collected from Akola and Buldhana districts, Maharashtra, India, during 199798. The collected seeds carried *Alternaria tenuis* (*Alternaria alternata) Bipolaris sorokiniana, Curvularia lunata (Cochliobolus lunatus), Drechslera sacchari (Bipolaris sacchari), Tricothecum roseum, Fusarium* spp., *Penicillium* spp*., Rhizopus* spp. and *Aspergillus* spp. Among the fungi, Bipolaris sorokiniana was the most predominant with the highest incidence (80.5% seeds).

Wenlan and Guozhen (2001) isolated some 4001 parasitic fungi from 4500 seeds of 30 wheat cultivers in eastern Hebei, China.They found *Alternaria triticina*, *Alternaria alternata, Bipolaris sorokiniana, Fusarium g*

rminearum (Gibberella zeae),Drechslera tritici and *Phoma leveillei* were pathogenic to seeds, stems and seedling of wheat.

Dhruj and Siddiqui (2000) reported *A. alternata* as the most predominant fungus followed by *Drechslera sorokiniana (Cochliobolus sativus*) in 168 affected seed lots tested in India. Other fungi occuring less commonly included *Curvularia cladosporioides. Drechslera tetramera (Cochliobolus spicifer), Drechslera halodes (Setosphaeria rostrata), Fusarium moniliforme (Gibberela fujikroi), Fusarium semitectum (Fusarium pallidorosem), Curvularia lunata (Cochliobolus lunatus), Trichothecium roseum, Nigrispora* sp., *Ulocladium* sp., *Stemphylium* sp. and *Verticillium* sp.

Hossain (2000) identified that *Alternaria tenuis, Bipolaris sorokiniana*, *Curvularia lunata* and *Fusarium* spp. were associated with wheat seed. He reported that the incidence of *Bipolaris sorokiniana* increased with the increase in level of infection and severity of black point infections.

Mondol and Sarandon (2000) reported that seed infection by *Bipolaris sorokiniana* results germination failure, seedling mortality and spot blotch development in wheat.

Sisterna *et al*. (2000) found that blackpoint was characterized by a brown to black discoloration of wheat kernels and occurs in all major wheat growing regions of the world. This disease was caused primarily by either

Alternaria alternata or *Bipolaris sorokiniana*. To identify the organisms associated with the disease seed health testing was carried out using the "blotter test" method. *Alternaria alternata* was found in most of the samples analyzed, followed by *Bipolaris sorokiniana*, the later showing very low infection levels. Other organisms were also found. Disease incidence differed between years and locations and none of the cultivars was completely free of the disease.

Shabana *et al.* (2000) reported that a total of 22 seed borne fungal species were isolated by blotter and agar plate methods from wheat seed collected from 9 districts in Rajasthan, India. The most predominant species, which were identified in samples from the 9 districts, were *Alternaria alternata*, *Alternaria tenuissima, Alternaria triticina* and *Alternaria triticola*.

Fernandez *et al.* (1998) studied the identification of the cause of discoloration of wheat seeds. They found infection of wheat kernels by *Pyrenophora tritirepentis* causes primarily a pink/ red discoloration. Dark smudge and black point are also commonly observed. These types of kernel discoloration cause downgrading of wheat seeds.

Ilyas *et al*. (1998) reported that the seeds of 15 wheat cultivers were assessed for apparent black point infection by visual observation and 5 seed-borne fungi, *Alternaria tenuis, Helminthosporium sativum*, *Curvularia lunata*, *Aspergillus* and *Penicillium* spp. were detected by the

blotter method. *Alternaria tenuis* was found to be the predominant seed-borne fungus.

Liu *et al*. (1998) concluded that the results of 4 yrs studies showed that black spot of wheat commonly occurred in Henan Province, China. The chief cause of black spot was *Alternaria tenuis* with a frequency of occurrence of 76.1%. *Alternaria tenuissima* was the second most detected pathogen (14.1%) followed by *Bipolaris sorokiniana* (7.0%), *Curvularia sativus* was the most pathogenic. It was first discovered that *Alternaria tenuissima* was also an important causal agent in China.

Rahman and Islam (1998) stated that 1000-grain weight of black point affected seeds decreased with increasing level of disease severity.

Zhimin *et al.* (1998) reported that seed germination and seedling growth decreased with the increase in susceptibility to a variety of infection.

Bazlur Rashid (1997) reported that highly significant effect of seedborne infection by *Bipolaris sorokiniana* on the germination of seeds of wheat cvs. Kanchan and Sonalika was recorded by rolled paper towel germination test as well as in pot experiment. At the maximum seedborne infection level (90%) both the cultivars yielded the minimum germination of 30.25 and 26.50% respectively. Relationship between the levels of seedborne infection and present seed germination showed gradual reduction in germination of seed with the increase of infection

level. There was a trend of decrease in seed germination with the increase in seedborne infection in both the cultivars. The maximum germination reductions were found as 71.50% and 68.00% in cv. Sonalika and cv. Kanchan, respectively.

Rashid *et al*. (1997) conducted an experiment and found that *Bipolaris sorokiniana* was associated with the surface of the wheat seeds.

Al-Rokibah (1996) conducted a survey of 65 fields of spring wheat cultivar and found seeds were infected with *Alternaria, Helminthosporium, Fusarium, Aspergillus* and other fungi at levels of 48, 20.1, 20.1, 3.9 and 3.8%, respectively.

Santorelli and Puglia (1996) stated that a total of 85 samples of each of durum wheat and soft wheat produced between 1991 and 1993 in different areas of Italy were analyzed for the presence of fungal pathogens. With the exception of some samples having a high presence of *Bipolaris sorokiniana* (*Cochliobolus sativus*), *Fusarium* spp. and *Microdochium nivale (Monographella nivalis*) were the most frequent causes of contamination or infection of seeds.

Dhruj and Siddiqui (1994) reported that *Alternaria tenuis* was predominant in the North Western Plain Zone and *Drechslera sorokiniana* in the North Eastern Plains of India.

Khan and Bhutta (1994) studied that seed-borne mycoflora of 25 wheat cultivars were investigated in Pakistan during 1985-90. The main pathogen isolated was *B. sorokiniana (Cochliobolus sativus*) followed by *Fusarium moniliforme (Gibberella fujikuroi) and Cephalosporium acremonium (Acremonium strictum*). Other pathogens isolated included *Penicillium*

Fusarium semitectum (Fusarium palladoroseum),

Alternaria spp and *Aspergillus* sp.

Agarwal *et al*. (1993) reported that grains with dark brown to black discoloration, generally restricted to the area around the embryo (typical black point symptoms), showed 100% infection by *Alternaria alternata*. Wheat grains with light brown to dark brown discrete lesions and a dull white spherical or elliptical area in the centre (typical 'eye-spot' symptoms) demonstrated infection by *Drechslera sorokiniana* alone. Grains with a creamy white or pinkish color, mostly shriveled and lighter in weight were infected by *Fusarium graminearum*. Certified wheat seeds, apparently healthy looking have been reported to be infected (35 - 37%) by *Drechslera sorokiniana.*

Alam *et al*. (1993) isolated 27 fungal species (14 genera) from wheat seed collected from different localities of which the most frequent fungi were *Alternaria nigar* and *Alternaria alternata* and the least frequent were *Fusarium* spp.and *Curvularia lunata* (*Cochliobolus lunatus*).

Moslem *et al.* (1993) observed that fungi were isolated from seeds of lentil, barly and wheat collected from a market in Riyadh, Saudi Arabia, using the standard blotter and agar plate methods. The predominant genera *Aspergillus* (10 species), *Curvularia* (7 species), *Alternaria* (5 species), *Drechslera*, *Penicillium* and *Trichosporon* (4 species each).

Languasco *et al*. (1993) observed that in Central Italy *Alternaria alternata* was identified as the predominant cause of black point and was isolated from 14.5% of grains. *Drechslera sorokiniana* was isolated from 0.3% of the wheat grains.

Ali and Fakir (1992) identified 16 fungi fewer than 9 genera occurring in wheat grains collected from different research stations of BARI. The most dominant fungi in order of prevalence were *Alternaria tenuis, Bipolaris sorokiniana, Aspergillus* spp., *Curvularia lunata* and *A. flavus.*

Bazlur Rashid (1992) studied that when 103 seed samples were tested by the freezing blotter method, 8 species of *Bipolaris* were isolated, of which the commonest was *Bipolaris sorokiniana*. Seed-born infection was highest in cv. Sonalika seed collected from Mymensingh (27.4%) and Meherpur (25.7%) and lowest (1.5%) in cv. Kanchan seed from Pabna.

Hyder-Ali and Fakir (1992) reported that sixteen fungi representing nine genera, were detected from seeds of seven cultivers of wheat collected

from Gazipur, Ishurdi, Jamalpur, Jessore and Mymensingh. Bipolaris sorokiniana was found one of the most predominant fungi.

Hossain (1991) studied the that severity of leaf blight of wheat caused by Bipolaris sorokiniana (*H. sativum*), on 533 wheat germplasms of local and exotic origin under field conditions and none was found to be free from infection by the pathogen in Bangladesh conditions.

Rosas (1991) observed that Germination, plumule growth, conductivity and seed health tests were used to study the effect of *Fusarium* spp. and *Septoria nodorum* infection of wheat. Results showed that seed inoculation with this pathogens reduced seedling growth, but did not affect percentage germination.

Shaarawy *et al.* (1991) studied that wheat cv. Kanchan 69 seeds from different locations in Egypt were assessed for seed injury (including discoloration, poorly filled seeds, shrunken seeds and broken seeds), seed born fungi, percentage germination and occurrence of abnormal seedlings. The percentage abnormal seedlings was 3-4% in discolored and mechanically damaged seeds and 1-2% in reduced shrunken seeds. The highest percentage of fungi were isolated from completely discolored (brown) seeds and from poorly filled seeds.

Fakir *et al*. (1989) observed the effect of five black point fungi on germinating seeds and emerged seedling when naturally infected wheat seeds were incubated by blotter method and seedling symptoms test, respectively. Among the rest fungi, *Drechslera* and *Fusarium* were found pathogenic, capable of causing germination failure /seed rot and or infection to the emerged seedling in both the methods. The formers caused more seedling infection.

Kunwar (1989) observed that fungi associated with stored wheat grains were isolated from samples collected from several locations in India. *Aspergillus* spp. were isolated from all the samples, whereas *Penicillium*, other imperfect fungi and Mucorales were found in 83, 62 and 50% of the samples, respectively.

Singh *et al.* (1989) reported *Alternaria alternata* as major pathogen in Western and *Drechslera sorokiniana* in Eastern part of India. Other fungi associated with black pointed seeds were *Alternaria triticina, Drechslera tetramera, Drechslera hawaiiensis, Curvularia lunata, Curvularia pallescens, Curvularia geniculata, Fusarium semitectum* and *Fusarium moniliform.*

Corner and Kuzyk (1988) and Madariaga and Mellado (1988) stated that *A. tenuis* was predominant in 80-90% seed samples, while *Helminthosporium sp.* and *Fusarium* sp. were observed only in few samples.

Fakir (1988) studied in Bangladesh the pathogenic nature of *Drechslera sorokiniana* and *Fusarium* species and stated that the fungi were found to cause germination failure/seed rot and disease to the emerged seedling.

Maloy and Specht (1988) isolated *Helminthosporium* and *Fusarium* from black pointed seeds of irrigated wheat in central Washington.

Agarwal *et al*. (1987) stated that *Alternaria alternate, Cladosporium oxysporum, Curvularia lunata, Bipolaris sorokiniana* and *Drechslera tetramera* were predominant fungi isolated from black point affected seeds.

Khanum *et al*. (1987) studied that high percentage of grains fails to germinate in the field due to fungi. The germination of healthy grains was 55-96.5% and that of diseased grains 34.5-71%. At least 5 fungal species were involved in the diseases complex in which *Alternaria alternata* was the most important.

Saari and Prescott (1986) were in the opinion that at least three different fungi possibly causing black point of wheat seed. *Alternaria tenuis* is not harmful at all and does not affect the seed germination, only darkly discolors the embryo end of the seed. *Drechslera sorokiniana* causes

some reduction in seed germination and also causes discoloration of embryo end of seed, while *Fusarium* spp. Causes whitish to pinkish discoloration of such grains.

Mehta and Igarash (1985) reported that the spot blotch of wheat caused by *Cochliobous sativus (Bipolaris sorokiniana*, syn *Helminthosporium sativum*) as one of the most important diseases in a number of countries such as Brazil, Paraguay, Bolivia, India, Bangladesh and Thailand.

Saari (1985) found that *Helminthosporium* leaf blight caused by *Helminthosporium sativum*, also known as *Bipolaris sorokiniana* and *Drechslera sorokiniana* with the perfect stage *Cochliobolus sativus* was a serious and sometime limiting factor to wheat and barley cultivation in tropical environment. Severe leaf blight, spike and the seed infections were common. The amount of spike or kernel infection in the tropics can be significant. If severe leaf infection is present and some rain occurs after heading the percentage of grain infection may exceed 50%.

Chaudhary *et al.* (1984) studied the effect of black point disease on germination of the grains of WL 711. The germination of the diseased seeds both in blotter method and in pots was reduced to 11.6 and 16.0% respectively. The invasion of pathogen on plumule and coleoptile might be impairing the germination, as lesions have been noticed in the young plumule and protruding out from diseased seeds. Reduction in germination to 44.67% has been observed on some cases.

Rees *et al*. (1984) stated that Seed germination was not affected by black point but seedling emergence was reduced by 3.2% from black-pointed seed.

Bazlur Rashid (1983) reported that leaf blight of wheat caused by *Drechslera sorokiniana* was investigated in Bangladesh. As much as 100 percent wheat plants were found infected by the diseases. *Bipolaris sorokiniana* was found pathogenic to wheat seedlings.

Bazlur Rashid *et al*. (1983) investigated on the Leaf blight of wheat caused by Drechslera sorokiniana was in Bangladesh over a period of four years (1976-80) in seven different districts of the country and as much as 100 percent wheat plants were found infected by the disease. They also found the disease as the most common and prevalent in wheat in the country in recent years.

Fakir *et al* (1977) established the seed to plant transmission of *Drechslera sorokiniana* in wheat. They stressed the importance of introduction of seed health testing programme for wheat in Bangladesh.

Adlakha and Joshi (1974) reported that if the infection of black points become severe the whole wheat grains becomes discolored and shriveled.

Vir (1974) stated that the seed borne infection of *Helminthosporium* was responsible for blight disease of wheat, barley, oat, rice and few other crops.

Mishra *et al*. (1969) found *Bipolaris sorokiniana* as the most economically important seed-borne and foliar pathogen of certain duram and aestivum wheat.

CHAPTER 3

MATERIALS AND METHODS

3.1 Experimental site

The experiments were conducted in the Laboratory of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.

3.2 Experimental period

This study was conducted during the period of May to November 2016.

3.3 Collection of seeds

A total of 20 seed samples of wheat (*Triticum aestivum L.*) were collected from different location of Kushtia, Jessore and Jhenaidah districts of Bangladesh. The size of each sample was 500 g (approx.). The seeds were then kept in poly bags and stored in the refrigerator at 5-7°C, till these were used for the subsequent studies.

3.3.1. Sources of collected wheat seed

Twenty samples of wheat (*Triticum aestivum* L.) seeds were collected from different upazilla of Kushtia, Jessore and Jhenaidah.

Table 1. Sources of collected wheat seed

Sample no	Variety	Date of collection	Area of Collection		
			District	Thana/upazilla	Village
1	Shatabdi	15/4/16	Kushtia	Durgapur	Magura
2	Shatabdi	8/4/16	Jessore	Sharsha	Baganchara
3	Prodip	20/4/16	Jessore	Keshobpur	Baro Mail
4	Prodip	20/4/16	Jessore	Keshobpur	Baro Mail
5	Prodip	20/4/16	Jessore	Keshobpur	Baro Mail
6	Prodip	20/4/16	Jessore	Keshobpur	Baro Mail
7	Shatabdi	21/3/16	Kushtia	Bheramara	Dharmapur
8	Shatabdi	29/3/16	Jhenaidah	Shailkupa	Fazilpur
9	Shatabdi	2/4/16	Jhenaidah	Shailkupa	Umedpur
10	Sonalika	15/4/16	Jhenaidah	Shailkupa	Dohokola
11	Sonalika	5/4/16	Jessore	Manirampur	Nehalpur
12	Shatabdi	1/4/16	Kushtia	Daulatpur	Daulatpur
13	Shatabdi	13/4/16	Kushtia	Daulatpur	Pearpur
14	Sonalika	23/3/16	Jessore	Manirampur	Rajgarh
15	Sonalika	23/3/16	Jessore	Manirampur	Shyampur
16	Prodip	23/3/16	Jessore	Jhikorgasa	Mamarakhpur
17	Prodip	1/4/16	Jessore	Chaugachha	Chaugachha
18	Shotabdi	18/4/16	Jhenaidah	Kaligonj	Baliadanga
19	Shotabdi	8/4/16	Jessore	Sharsha	Baganchara

20	Shotabdi	8/4/16	Jessore	Sharsha	Baganchara

3.4 Identification of seed-borne fungi associated with wheat seeds

All the seed samples were assayed for the presence of fungal pathogens by the Standard Blotter Method following the International Rules for Seed Testing Association (ISTA, 2004).

3.4.1 Evaluation of seed health status of wheat by blotter method

Seed health status was assayed by Blotter method to detect the seed borne pathogens in the samples. In this method, two hundred seeds were randomly taken from each sample. The seeds were plated on water soaked three layered Whatman No. 1 filter paper in plastic petridish. In each petridish, 25 seeds were plated at equal distance (Fig. 1). All these petridishes were incubated at 20±2°C under 12 hours alternate cycle of Near Ultra Violet (NUV) light and darkness. After 7 days of incubation, incubated seeds were observed under stereomicroscope for detecting seed borne fungi in Wheat seed surface under stereomicroscope at 25X magnification. Where identification was difficult or doubtful under the stereomicroscope, temporary slide was prepared and examined under the compound microscope and identified with the help of keys (Ellis, 1971 and Chidambaram et al., 1973).

Number of germinated seeds were recorded along with the seed-borne fungi after seven days of incubation. The results were expressed in percentage.

Fig.1. Wheat seeds in Blotter method

3.4.3 Rolled Paper Towel Method

The method developed by Warham (1990) was followed. Germinability of the seeds were determined in the laboratory at room temperature. (30±2°C). Two hundred seeds were randomly taken from each sample and 40 seeds were placed between a pair of moist paper towels . There were three replications for each sample. The towels were rolled and the ends were closed by threads and covered by polyethylene paper to prevent drying. Good quality towel paper (46 × 29 cm) free from toxic substance was taken for experiment. Sterilized water is used for moisten of the towel and one towel was placed over the seed. The towel paper

was rolled in such a manner that seeds remain in place (fig. 2). 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 and (f) Vigor Index. 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 of Abdul Baki and Anderson (1972)

Vigor Index = (Mean of root length + Mean of shoot length)

x percentage of seed germination.

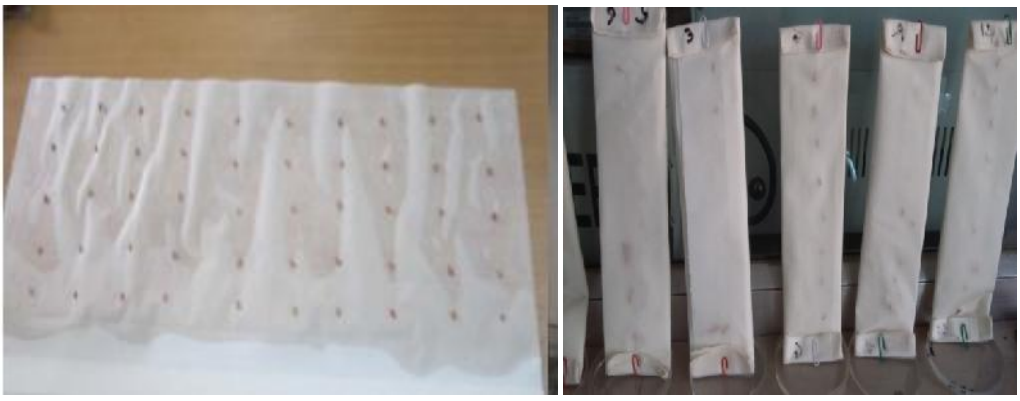

Fig.2. Rolled paper towel method

3.4.4 Seedling symptom test

This method was performed to determine seed borne pathogen transmission from seed to the seedlings of wheat. A required amount of 1% water agar was prepared and autoclaved. Approximately 15ml of water agar were poured into test tubes using an automatic dispenser and the test tubes autoclaved at 121 o C and pressure of 15 psi for 15 minutes. Agar was allowed to solidify by tilting the test tubes so as to get the required slant of the agar medium (approximately 30 oC from the horizontal) to facilitate examination of fungi growing. The seeds were surface sterilised in 0.5 % sodium hypochlorite for 2 - 5 minutes, rinsed in distilled water, blot dried and one seed dropped into each tube (Fig. 3). The tubes were incubated under 12 hour alternating cycles of NUV light and darkness for at least 7 days. Each sample had a ten test tubes.

Fig.3.Water agar seedling symptom test

3.4.5 Deep Freezing Method

The provided samples were used for the isolation and detection of seed-borne fungi. The Deep Freezing Method (Limonard 1968) was recommended for the isolation of the fungi. Isolation was made from 200 seeds of each sample under aseptic conditions. Twenty five seeds per plate were placed on three layers of moistened blotters (Fig. 4). The seeds were incubated for one day at 25±1°C followed by 12 hr/12hr alternative cycle of NUV .

Fig.4. Deep freezing method

3.5 Analysis of data

The design of experiment was CRD (Completely Randomized Design). The recorded data on various parameters under the present study were statistically analyzed using MSTAT statistical-package programme. The

level of significance and analysis of variance along with the Least Significant Difference (LSD) was done (Gomez and Gomez, 1984).

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Determination of occurrence of seed borne fungi of wheat seed collected from different location

After seven days of incubation on blotter paper the growing fungi were observed under microscope then identified. Significant variations among the sample in respect of percent seed germination and incidence of seed borne fungi were observed in Blotter paper method (Table-02). In case of germination, significant variation observed among the sample. The highest seed germination was recorded in variety Shatabdi (1) (66.40%) followed by Prodip (3) (48.23%). And the lowest germination was recorded in sample no Shatabdi (2) (2.73%). Eight fungal species viz. *Bipolaris sorokiniana , Aspergillus flavus, Aspergillus niger, Alternaria tenuis, Fusarium moniliforme, Penicillium* spp*., Curvularia lunata* and *Pyricularia grisea* were observed (Fig. 6-13).

The incidence of *Penicillium* spp. ranged from 4.oo to 15.33% where the highest incidence was recorded in Prodip 6 and lowest incidence was observed in sample Shotabdi (1), Shotabdi (5), Prodip (1), and Prodip (3). The highest incidence 34.00% of *Aspergillus flavus* was recorded in Prodip where the lowest incidence was 10.33% recorded in Prodip(5) . The incidence of *Aspergillus niger* ranged from 5% to 20%. The highest incidence was recorded in Prodip (1) (20%) where the lowest incidence was recorded in Shotabdi (1). The incidence of *Bipolaris sorokiniana*

ranged from 3.00 to 17.67 % where the highest incidence was in Shotabdi (6) (17.67%) and lowest incidence was in sample no Prodip (2) (3.00%). The incidence of *Fusarium moniliforme* was varied from 4.00 to 17.61% while the highest incidence was recorded in variety Shotabdi (6) (17.61%) followed by sample no Shotabdi (10) (14%). The incidence of *Alternaria tenuis* was varied from 5.00 to 14.33% and the highest incidence was recorded in Shotabdi (10) (14.33%) followed by variety Sonalika (1) (12.67%) and lowest incidence was in Prodip (1) and Shatabdi (2) (5.00%). The incidence of *Curvularia lunata* was varied from 2.33 to 12.67% while the highest incidence was recorded in variety Shotabdi (2) (12.67%) followed by Prodip (6) (7.33%) and lowest incidence was in Prodip (1). The incidence of *Pyricularia grisea* was varied from 0 to 5.33% and the highest incidence was recorded in Shotabdi (2) and lowest incidence was in sample no Shotabdi (7).

A considerable number of seed-borne fungal pathogens belonging to the genera *Bipolaris, Alternaria, Curvularia, Fusarium, Penicillium* and *Aspergillus* have been detected in wheat seeds as reported by many researchers Singh et al. (2011), Hajihasani et al. (2012), Pathak and Zaidi (2013b), Zrari (2013) and El-wakil (2013).

Table 2.% Germination and prevalence of seed borne fungi of wheat seeds on Blotter

Variety	Germination %	*Penicillium sp*	*Alternaria tenuis*	*Aspergillus flavus*	Aspergillus niger	*Fusarium monilifome*	*Bipolaris sorokiniana*	*Curvularia lunata*	*Pyricularia grisea*
Shatabdi(1)	66.40a	4.00 h	11.00 bc	19.67 de	5.000 h	4.00 m	17.33 ab	6.000cde	2.00 d
Shatabdi(2)	2.73 l	4.66 gh	5.000 g	26.67 b	14.67 b	12.0def	5.667i	12.67 a	5.33a
Shatabdi(3)	27.33 d	5.67 fgh	9.330 cde	20.67 d	13.00 cd	9.00 ij	11.00 h	6.00cde	1.00 g
Shatabdi(4)	21.33 fgh	8.00 de	10.00 cd	23.33 c	8.670 g	6.67 kl	13.00 fgh	4.00 i	**4.00 b**
Shatabdi(5)	12.33 j	4.00 h	9.000 de	19.00 def	7.670 g	6.00 l	14.67defg	5.67 def	0.33 i
Shatabdi(6)	26.03 de	12.0 c	7.000 f	17.67 ef	11.67 def	9.00ij	17.67 a	4.67 ghi	0.67 h
Shatabdi(7)	17.10 i	6.00 fg	8.670 de	21.00cd	11.67 def	9.00 ij	13.67 efg	5.67 def	0.00 j
Shatabdi(8)	18.70 hi	11.67 c	11.00 bc	16.67fg	13.67 bc	16.0 b	13.67 efg	5.33 efg	0.67 h
Shatabdi(9)	22.00 fg	14.00 ab	12.00 b	14.33 gh	13.33 bc	10.3 ghi	12.67 gh	6.67 bc	1.00 g
Shatabdi(10)	24.10 ef	8.670 d	14.33a	18.67 def	13.33 bc	14.0 c	15.33 bcde	4.67 ghi	0.33 i
Prodip(1)	34.67 c	4.00	5.000 g	34.00a	20.00a	8.00 jk	7.000i	2.33 j	2.00 d
Prodip(2)	4.667 l	5.00 gh	11.00 bc	18.67 def	11.00f	11.0 efgh	3.000 j	5.33 efg	1.33 f

Prodip(3)	48.23 b	4.00 h	9.000 de	17.67 ef	9.000 g	7.00 kl	12.67 gh	6.00cde	2.00 d
Prodip(4)	21.80 fg	6.67 ef	9.000 de	26.33 b	9.000 g	12.3de	13.00 fgh	4.33 hi	1.67 e
Prodip(5)	22.00 fg	13.67 b	11.00 bc	10.33 i	13.00 cd	13.3 cd	13.67 efg	5.67 def	0.67 h
Prodip(6)	20.00 ghi	15.33 a	7.667 ef	13.33 h	13.33 bc	17.6a	16.33 abcd	7.33 b	1.00 g
Sonalika(1)	14.00 j	8.67 d	12.67 b	26.67 b	13.33 bc	9.66 hi	14.00 efg	5.00 fgh	3.00 c
Sonalika(2)	8.66 k	9.00 d	12.00 b	18.00 ef	11.33 ef	11.0 efgh	17.00 abc	4.00 i	1.33f
Sonalika(3)	13.33 j	9.33 d	9.670 cd	16.67fg	12.67cde	10.6 fgh	14.33 defg	6.33cd	1.00 g
Sonalika(4)	23.67 ef	11.00 c	12.00 b	13.33 h	8.000 g	11.6 efg	15.00cdef	4.67 ghi	0.67 h
$LSD_{0.05}$	2.78	1.47	1.53	2.36	1.31	1.39	1.88	0.851	0.19
Level of significance	**	**	**	**	**	**	**	**	**
CV (%)	7.52	10.82	9.48	7.29	6.82	8.13	8.75	9.18	7.87

In a column, similar letter(s) do not differ significantly @ 1% level of probability

Fig.5. Showing germination test on Blotter paper

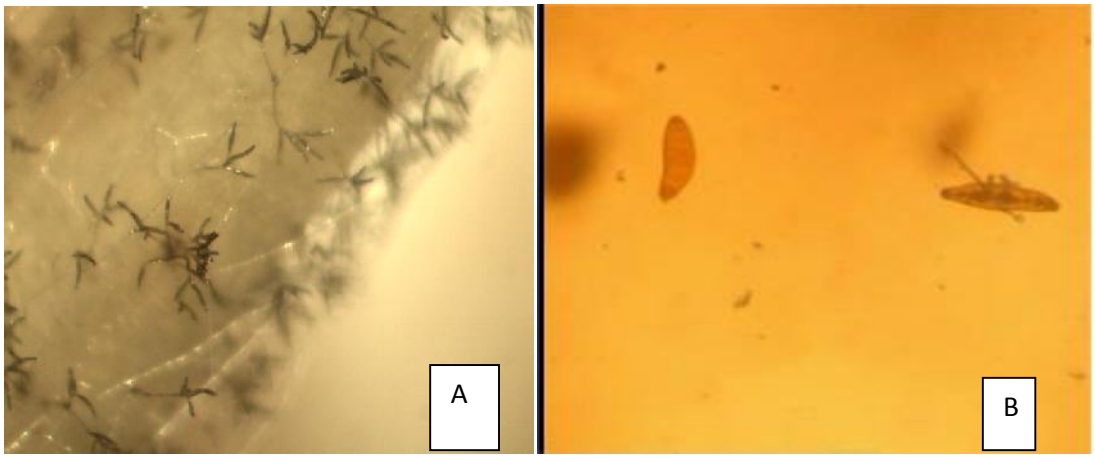

Fig.6. A. Stereo microscopic view (10X) and B. compound microscopic view (40X) of *Bipolaris Sorokiniana*

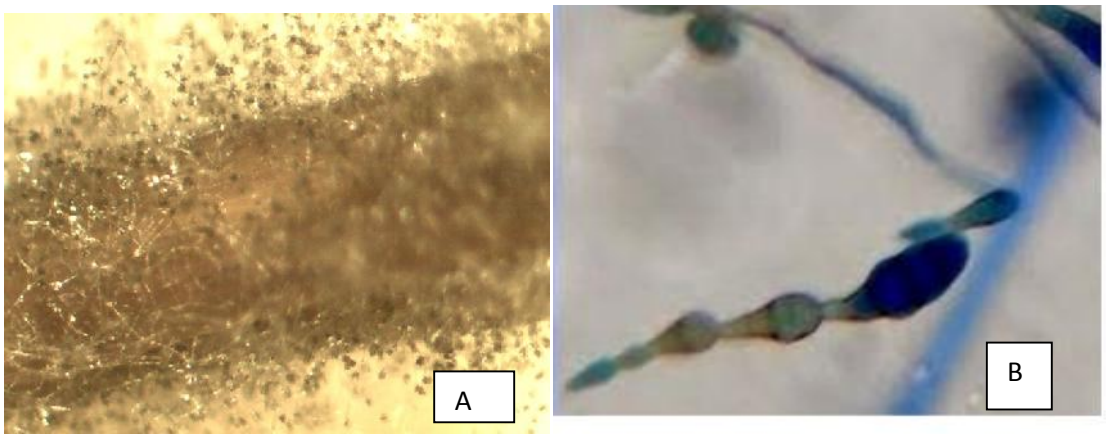

Fig.7. A. Stereo microscopic view (10X) and B. compound microscopic view (40X) of *Alternaria tenuis*

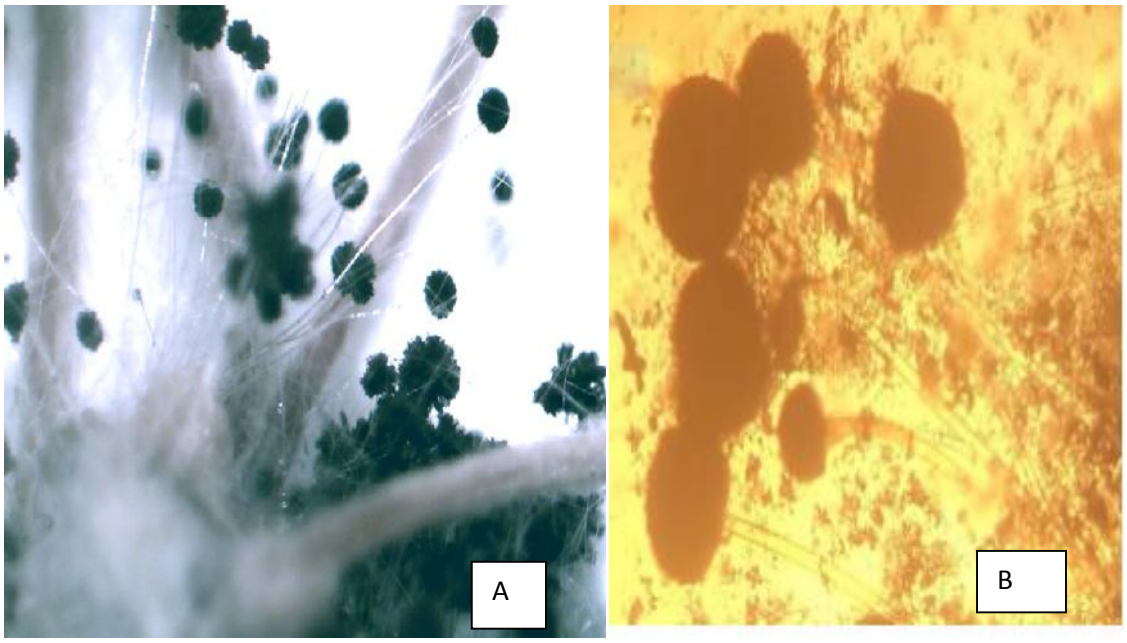

Fig. 8. A. Stereo microscopic view (40x) and B. compound microscopic view (10X) of *Aspergillus niger*

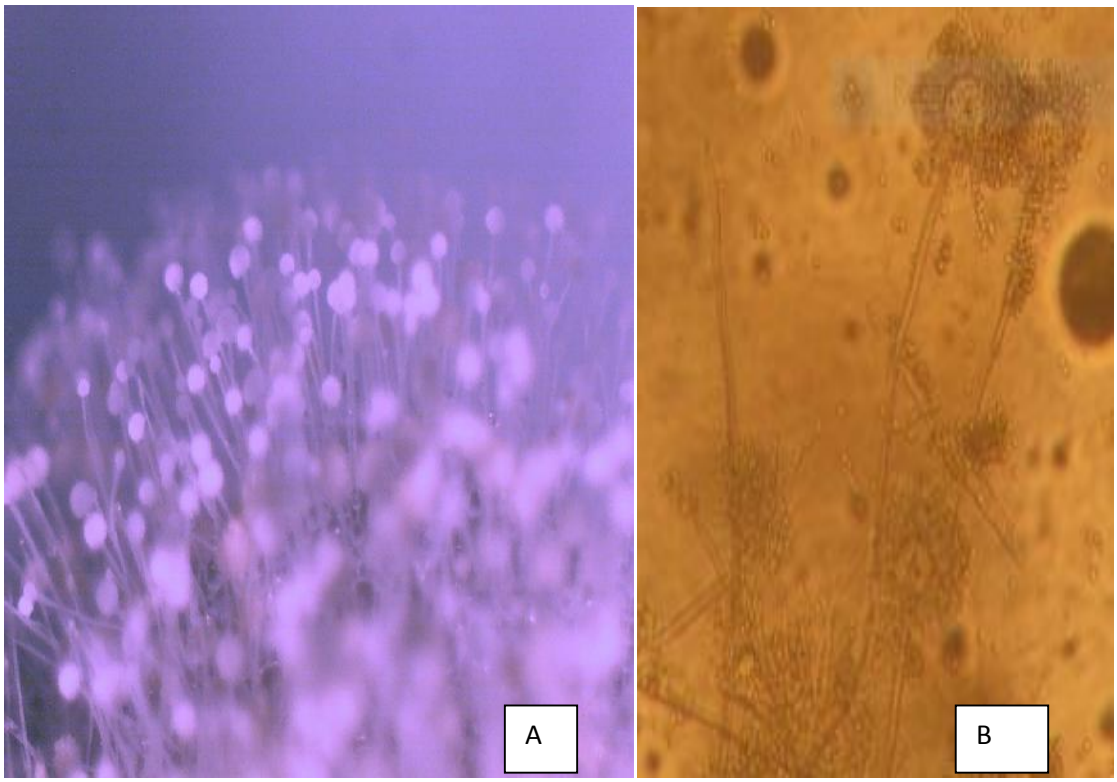

Fig. 9. A. Stereo microscopic view (10X) and B. compound microscopic view (10X) of *Aspergillus flavus*

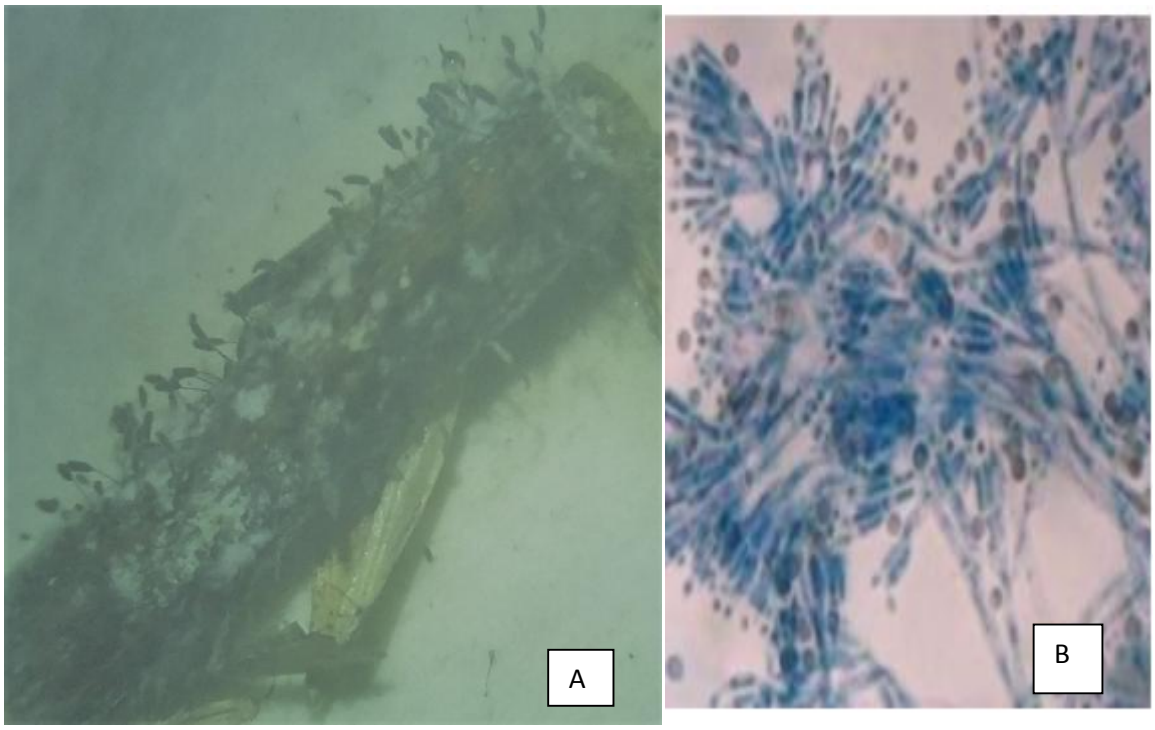

Fig. 10. A. Stereo microscopic view (10X) and B. compound microscopic view of *Penicillium* sp (40X)

Fig.11. A. Compounnd microscopic view (10X) of *Fusarium moniliforme*

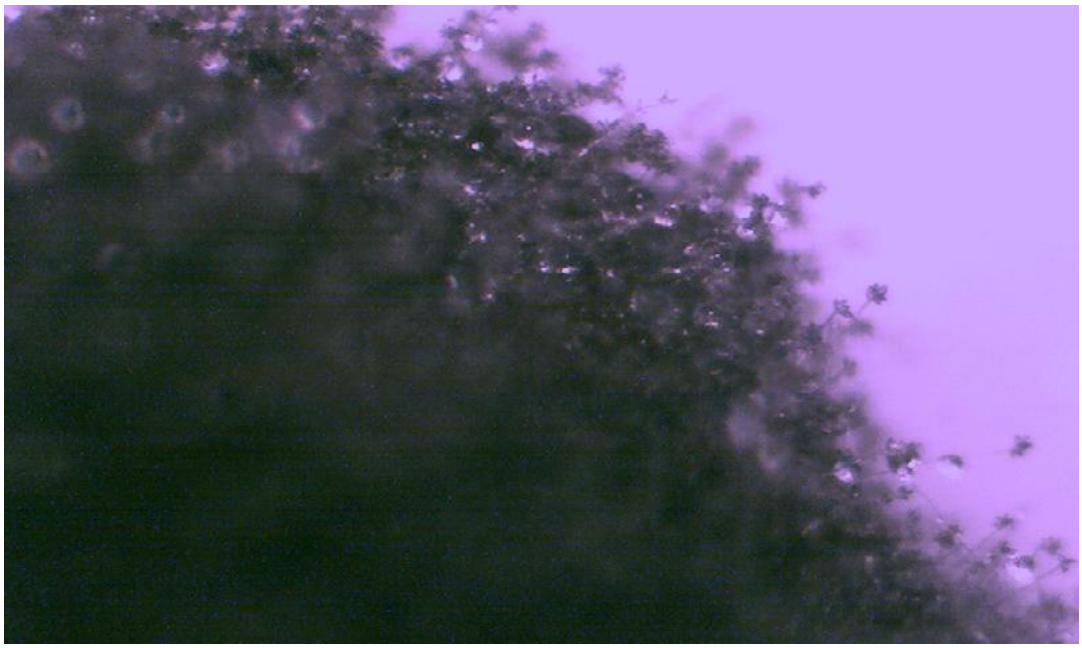

Fig.12. Stereo microscopic view (10X) of *Pyricularia grisea*

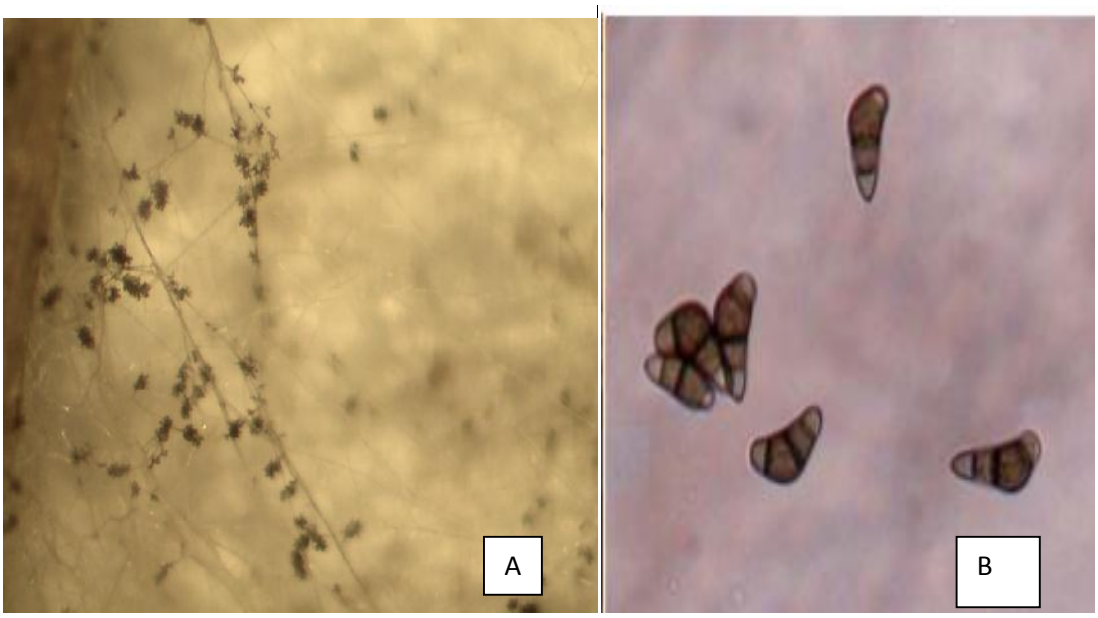

Fig. 13. A. Stereo microscopic view (10X) and B. compound microscopic view (40x) of *Curvularia lunata*

4.2 Effect of seed borne pathogens on seedling vigor (Rolled paper towel method)

Effect of seed borne pathogens on seedling vigor of collected sample of wheat were determined and significant results found regarding germination, seedlings weight, root length, shoot length and vigor index (Table 3). The highest seed germination percentage was recorded in variety Sonalika (1) (81%) and the

lowest germination percent (60%) was recorded in variety shotabdi (2) and shotabdi (10). The highest shoot length percentage were recorded in sample no variety prodip (4) and Sonalika (3) (14.00 cm) and the lowest was in variety shotabdi(2) (3.00%). The highest root length percentage was recorded in Sonalika (8) (16.67 cm) and the lowest was in shotabdi (2) (5.00cm).The seedling weight ranged 0.76-4.80 g per plant.The highest weight of seedling was recorded in variety Sonalika (1) (5.53g) where as the lowest was recorded in shotabdi (2) (0.76g). The vigor index of seedling was highest in sample no shatabdi (3) (19640) and lowest was in shotabdi (2) (480).The differences in this parameter at different levels of seed infected by pathogen were significant. Dharmvir, *et al*., (1968) also reported reduction in the germination of wheat seed due to fungi colonizing during storage.

Oppitz and Hoesser (1979) reported that seed borne pathogens of wheat not only reduced the germination but also affected seedling vigor resulting in low yield. Rees, *et al*., (1984) also recorded quality changes in wheat seed by *A. alternata*. Sulaiman and Husain (1984) observed that *Aspergillus flavus* reduced 90% germination of wheat seeds as compared to healthy seeds. Mahmuda (l987) detected *Alternaria alternata* to be predominant causing 82% reduction in germination of wheat seeds.

Table 3. Germination and seedling vigor of Wheat seeds or Rolled paper towel

variety	Germination (%)	Shoot length (cm)	Root length (cm)	Weight of seedling (g)	Vigor index
Shatabdi(1)	69.33 efgh	9.33 cde	13.67 c	3.483 efgh	1595. defgh
Shatabdi(2)	60.00 j	3.00 f	5.000 g	0.76 l	480.0 k
Shatabdi(3)	71.00 e	12.00 b	12.33 d	3.90 cdef	1964.a
Shatabdi(4)	65.33 hi	10.33 bcd	12.67 cd	3.25fgh	1728.bcde
Shatabdi(5)	54.67 k	9.66 cde	11.00 e	2.090 jk	1503. ghi
Shatabdi(6)	66.43 fghi	9.170 de	13.00 cd	3.217 gh	1579. efgh
Shatabdi(7)	71.43 e	10.33 bcd	12.67 cd	3.77 defg	1473. hi
Shatabdi(8)	66.00 ghi	10.67 bcd	16.67 a	3.31 efgh	1544. fgh
Shatabdi(9)	79.00 ab	11.00 bcd	11.17 e	4.23 bcd	1751. bcd
Shatabdi(10)	60.00 j	9.33 cde	12.33 d	2.21 jk	1360. i
Prodip(1)	70.47 ef	10.33 bcd	13.50 c	1.60k	480.0 k
Prodip(2)	77.67 abc	8.33 e	10.33 e	4.80 b	1679.cdef
Prodip(3)	66.00 ghi	10.83 bcd	12.33 d	3.48 efgh	1450. hi
Prodip(4)	73.67 cde	14.00 a	12.67 cd	4.30 bcd	1528. fgh
Prodip(5)	70.00 efg	14.00 a	12.33 d	3.060 hi	1567. efgh
Prodip(6)	76.10 bcd	10.00 cde	10.33 e	3.94 cde	1844. ab
Sonalika(1)	81.00 a	11.33 bc	10.83 e	5.53 a	1130. j

Sonalika(2)	77.00 abcd	12.17 b	8.330 f	3.46 efgh	1796.bc
Sonalika(3)	73.67 cde	14.00 a	12.67 cd	4.30 bcd	1528. fgh
Sonalika(4)	70.00 efg	14.00 a	12.33 d	3.060 hi	1567. efgh
LSD0.05	3.93	1.69	0.97	0.59	143.40
Level of significance	**	**	**	**	**
CV (%)	3.43	9.93	4.88	10.64	5.56

In a column, similar letter(s) do not differ significantly @ 1% level of probability

Fig.14. Measuring the shoot and root length of seeding

4.3. Effect of seed borne pathogens on seedlings (water agar method)

Germination and seedling (%) of collected sample were determined (Table 4). Significant variation was observed among the samples regarding germination,

abnormal seedling, normal seedling and dead seed. Incase of germination**,** the highest germination was recorded in variety Prodip (4) (70.00%) which was collected from Baro mail, Keshobpur, Jessore followed by variety Prodip (2) (66.67%). Incase of normal seedling, the number of normal seedlings was highest Sonalika (3) (46.67%) and lowest was in sample no Shotabdi (5) which was collected from Baganchara, Sharsha, Jessore. Incase of abnormal seedling, the number of abnormal seedlings was highest in Prodip (4) (50%) and the lowest was recorded in sample no Prodip (6) (20%). Dead seed percentage was recorded highest in variety Shotabdi (5) (66.67%) which is followed by sample no Shotabdi (2) and Prodip (1) (53.55%). On the other hand the lowest one was sample no Sonalika (3)(30.00%).

The present findings support the findings of Guerrero *et al.* (1972) and Islam *et al*. (2000). Guerrero *et al.* (1972) showed that most of the seed borne pathogens cause abnormal seedlings and Islam *et al*. (2000) observed that highest lethal seed infection caused by seed borne pathogens.

Table 4. **Germination and seedling (%) development on water agar of wheat seeds or seedling symptom test**

variety	Germination (%)	Normal Seedling (%)	Abnormal seedling (%)	Dead seedling (%)
Shatabdi(1)	40.00 efg	23.33 e	26.67 f	50.00cd

Shatabdi(2)	40.00 efg	10.00 i	36.67c	53.33bc
Shatabdi(3)	53.33 cd	23.33 e	36.67c	40.00fg
Shatabdi(4)	46.67 cdef	16.67 g	26.67 f	56.67b
Shatabdi(5)	36.67 fg	3.33 j	30.00 e	66.67a
Shatabdi(6)	53.33 cd	10.00 i	36.67c	53.33bc
Shatabdi(7)	56.67 bc	23.33 e	40.00 b	36.67gh
Shatabdi(8)	43.33 defg	13.33 h	30.00 e	56.67b
Shatabdi(9)	50.00 cde	16.67 g	40.00 b	43.33ef
Shatabdi(10)	53.33 cd	26.67 d	23.33 g	50.00cd
Prodip(1)	33.33 g	20.00 f	26.67 f	53.33bc
Prodip(2)	66.67 ab	43.33 b	30.00 e	26.67j
Prodip(3)	56.67 bc	33.33 c	30.00 e	36.67gh
Prodip(4)	70.00 a	16.67 g	50.00 a	33.33hi
Prodip(5)	40.00 efg	13.33 h	30.00 e	56.67b
Prodip(6)	50.00 cde	33.33c	20.00 h	46.67de
Sonalika(1)	53.33 cd	26.67 d	36.67c	36.67gh
Sonalika(2)	56.67 c	26.67 d	33.33 d	40.00fg
Sonalika(3)	73.33 a	46.67 a	23.33 g	30.00ij
Sonalika(4)	40.00 efg	13.33 h	30.00 e	56.67b
LSD0.05	9.82	1.41	1.90	3.71
Level of significance	**	**	**	**

CV (%)	11.75	3.82	3.67	4.87

In a column, similar letter(s) do not differ significantly @ 1% level of probability

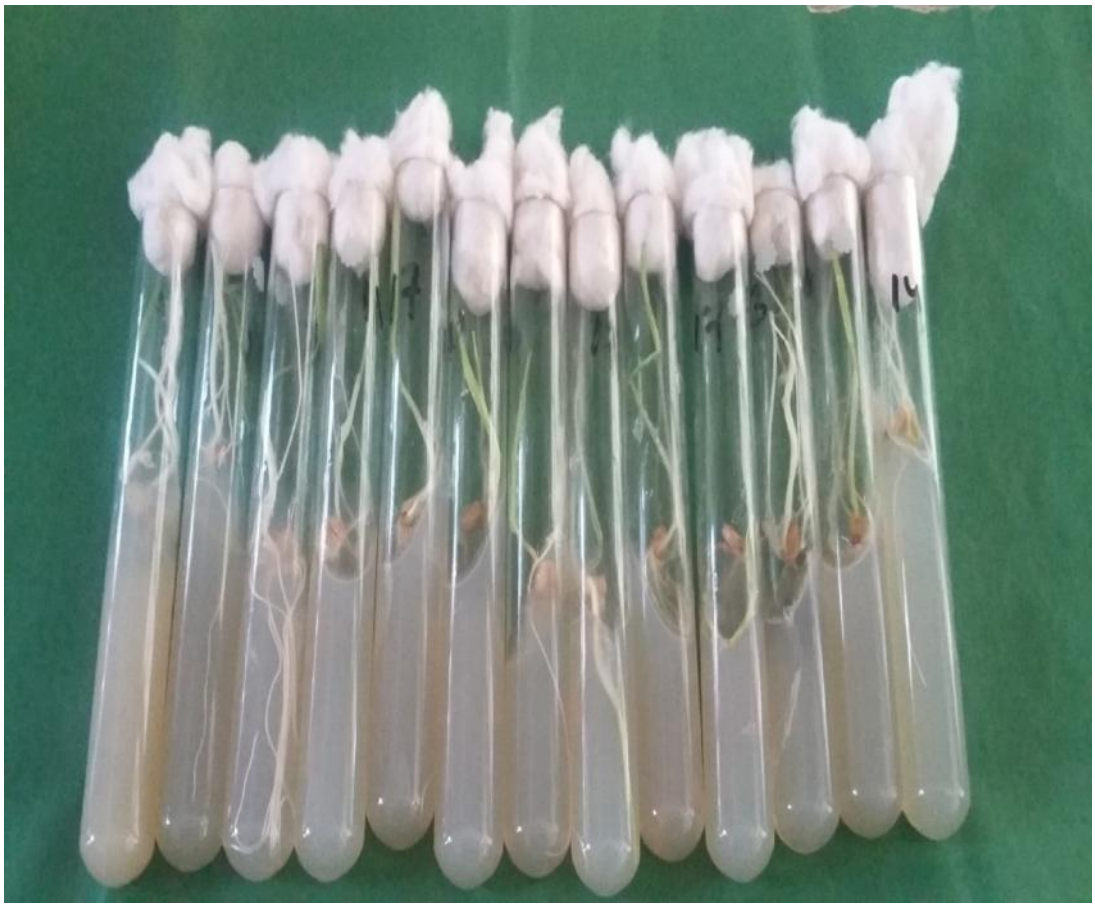

Fig.15. Seedling symptom test on water ager test tube method.

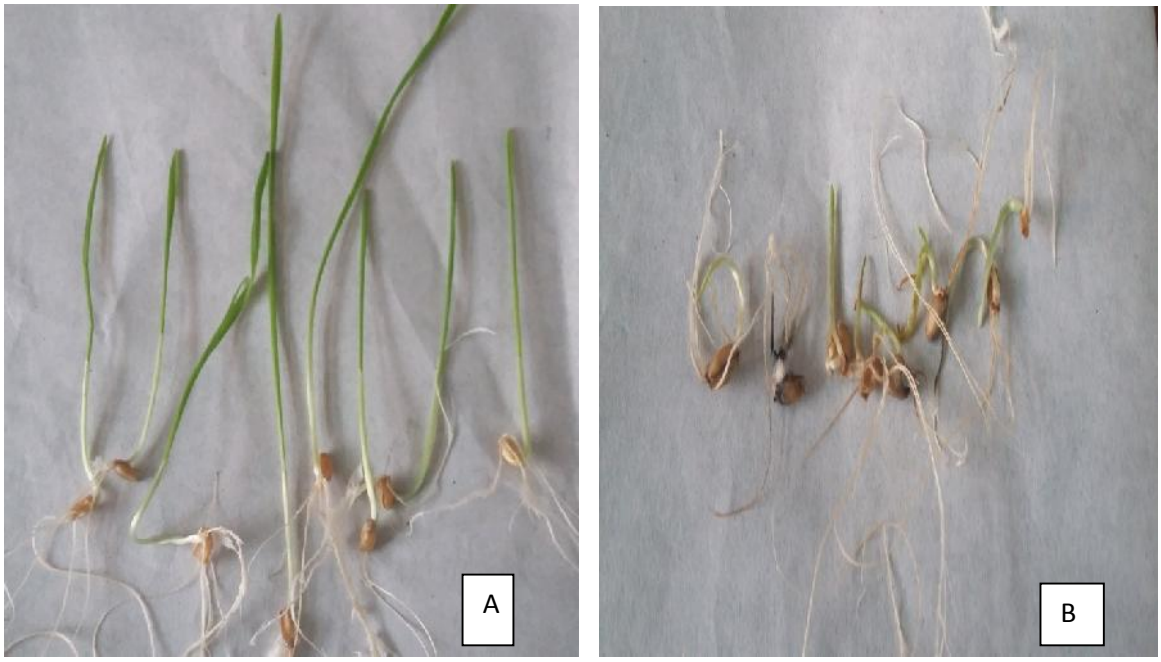

Fig.16. Normal (A) and Abnormal (B) seedling

4.4. Seed health study of collected seed samples using Deep freezing blotter method

Eight fungal species viz*. Bipolaris sorokiniana , Aspergillus flavus, Aspergillus niger, Alternaria tenuis, Fusarium moniliforme*, *Penicillium* spp., *Curvularia lunata* and *Pyricularia grisea* were observed.

Assawah and El-Arosi (1960), El-Kady *et al*. (1982) and Mazen *et al*. (1984) observed that *Aspergillus*, *Fusarium*, *Penicillium*, and *Rhizopus* were the most common genera in wheat grains in Egypt.

The incidence of *Penicillium* spp.ranged from 2.00 to 9.00% where the highest incidence was recorded in Shatabdi (3) and Shatabdi (5) (9.00%) and lowest incidence were observed in Shotabdi (2), Prodip(2) and Prodip (6). The highest incidence of *Aspergillus flavus* was recorded in sample Prodip (4) (7.00%) where the lowest incidence was recorded in Sonalika (3) (3.66%). The incidence of *Aspergillus niger* ranged from 2.00 to 8.00%. The highest incidence was recorded in variety Shotabdi (7) (8.00%) where the lowest incidence were recorded in sample no Shotabdi (10) and Prodip (6). The incidence of *Bipolaris sorokiniana* ranged from 14.00 to 30.00 % where the highest incidence was in Sonalika (2) (30.00%) and lowest incidence was in Sotabdi (10) (14.00%). The incidence of *Fusarium moniliforme* was varied from 11.00 to 21.00% and the highest incidence was recorded in Shotabdi (8) (21%) and the lowest were recorded in Prodip (1) and Prodip (6) (11%). The incidence of *Alternaria tenuis* was varied from 12.00 % to 23.00% and the highest incidence were recorded in Shotabdi (9) and sample no Shotabdi (5) (23.00 %) followed by Shotabdi (10) (21.00%) and lowest incidence was in Prodip (5) (12.00%) . The incidence of *Curvularia lunata* was varied from 3.67 % to 14.00% and the highest incidence was recorded in Shotabdi (9) and Sonalika (4) while lowest incidence was in Prodip (2) (3.67%). The incidence of *Pyricularia grisea* was varied from 0 to 4.00% and the highest incidence was recorded in variety Shotabdi (8) (4.00%) and lowest incidence was in sample no Prodip (5) and Sonalika (2) (0).

Many reports indicated the occurrence of many fungal genera in different wheat cultivars in other countries of the world. Included that *Alternaria, Helminthosporium, Fusarium, Curvularia, Stemphylium, Cladosporium, Aspergillus, Penicillium, Microdochium, Bipolaris, Mucor, Botrytis, Rhizopus, Aureobasidium, Dreschslera,* and *Rhizoctonia* according to (Bhutta and Hussain 1999; Rajput *et al*. 2005; Hassan *et al*. 2005; Javaid and Anjum 2006; Fakhrunnisa *et al*. 2006; Singh *et al*. 2011; Hajihasani *et al*. 2012; Hussain *et al*. 2013; Jalal and Zrari 2013; Majumder *et al*. 2013; and Pathak and Zaidi (2013b).

Ghosh and Nandi (1986) reported that several species of *Aspergillus* and *Penicilium* are responsible for the deterioration of wheat grains during storage. Pre and post-harvest biodeterioration and spoilage of grains due to infestation by microorganisms may cause losses of up to 100% (Satish *et al*. 2010). The species of *Aspergillus* has been reported to cause a significant loss in the seed quality and nutritional value of grains (Koirala *et al*. 2005).

Table 5. % Seed borne fungi of collected wheat seed samples using deep freezing blotter method

variety	*Penicillium* spp	*Alternaria tenuis*	*Aspergillus flavus*	*Aspergillus niger*	*Fusarium monilifome*	*Bipolaris sorokiniana*	*Curvularia lunata*	*Pyricularia grisea*
Shatabdi(1)	3.33 ef	12.33 hi	5.333 bcde	4.00 f	12.67 gh	19.67 e	7.00g	2.00 d
Shatabdi(2)	3.66 ef	15.00 efg	6.000 abc	4.66de	13.00 fgh	26.67 bcd	5.33 hij	1.67 e
Shatabdi(3)	9.00 a	19.00 c	5.000 cdef	4.33 ef	17.00bcde	18.00 ef	5.33 hij	1.67 e
Shatabdi(4)	3.00 f	21.00 b	4.000 fg	6.00 c	17.67bcd	25.00 d	5.66 hi	2.00 d
Shatabdi(5)	5.00 d	23.00a	4.670 defg	3.00 g	13.67 fgh	28.00 abc	5.00 ij	3.00 b
Shatabdi(6)	2.00 g	17.00 de	4.667 defg	6.00c	14.00 efgh	19.00 e	11.00c	1.00 f
Shatabdi(7)	5.00 d	15.00 efg	4.667 defg	8.00 a	18.00 bc	16.00 fgh	9.000e	3.00 b
Shatabdi(8)	7.00 b	17.00 de	5.000 cdef	4.00f	21.00a	15.00 gh	8.000 f	4.00 a
Shatabdi(9)	9.00 a	23.00a	6.000 abc	3.00 g	19.00ab	20.00 e	14.00a	3.00 b
Shatabdi(10)	5.00 d	21.00 b	7.000 a	2.00 h	12.00 gh	14.00 h	9.000 e	2.00 d
Prodip(1)	6.00c	16.33def	4.333 efg	4.00 f	11.00 h	18.67 ef	4.67 j	1.00 f
Prodip(2)	2.00 g	13.00 hi	6.000 abc	5.00d	13.00 fgh	17.67 efg	3.67 k	0.67 g
Prodip(3)	4.00 e	15.00 efg	4.000 fg	4.00 f	14.67efg	26.33 bcd	5.33 hij	1.67 e
Prodip(4)	7.00 b	15.00 fg	5.670 bcd	2.33 h	14.00efgh	19.00 e	5.66 hi	2.33 c
Prodip(5)	3.00 f	12.00 i	5.667 bcd	4.00 f	16.00cdef	24.00 d	12.00b	0.00 h

Prodip(6)	2.00 g	14.00 gh	5.333 bcde	2.00 h	11.00 h	26.00 cd	10.00d	1.00 f
Sonalika(1)	4.00 e	19.00c	5.670 bcd	5.00d	14.33 efg	24.00 d	6.00 h	1.00 f
Sonalika(2)	3.00 f	16.00 ef	6.333 ab	7.00 b	11.00 h	30.00 a	10.00 d	0.00 h
Sonalika(3)	6.00 c	17.00 de	3.667 g	7.00 b	13.00 fgh	29.00 ab	12.00 b	2.00 d
Sonalika(4)	7.00 b	18.00cd	5.333 bcde	6.00 c	15.00 defg	18.00 ef	14.00a	1.00 f
$LSD_{0.05}$	0.672	1.75	0.994	0.574	2.67	2.57	0.738	0.209
Level of significance	**	**	**	**	**	**	**	**
CV (%)	8.50	6.29	11.54	7.60	11.15	7.19	5.50	7.34

In a column, similar letter(s) do not differ significantly @ 1% level of probability

4.5 Comparative study of germination and seed borne fungi in different methods

Seed germination (%) varied from 2.73 - 66.40%, 79-60% and 73.33 - 33.33% in blotter method, rolled paper towel method and water agar test tube method respectively. In blotter method Shatabdi(1) showed the highest germination 66.40%. In roller paper towel method the highest seed germination percentage was recorded in variety Sonalika (1) (81)%. The lowest germination percent was recorded in variety shatabdi (2) and shatabdi (10). In case of water agar method the highest germination was recorded in variety Prodip (70.00%) which was

collected from Baro mail, keshobpur, Jessor. Eight fungal pathogenes were identified by the both blotter paper and deep freezing blotter paper method. The occurrence of *Aspergillus flavus* 26.33%, *Aspergillus niger* 20.00% and *Penicillium* spp 15.33%, were high in the blotter paper than the deep freezing blotter paper method. On the other hand *Alternaria tenuis* 23.00%, *Fusarium moniliforme* 21%, *Bipolaris sorokiniana* 30%, *Curvularia lunata* 14.00% and *Pyricularia grisea* 4% showed highest in deep freezing blotter method.

Pathak and Zaidi (2012) reported that blotter method was found to be the best media for the isolation of mycoflora whether borne externally or internally.

CHAPTER 5

SUMMARY AND CONCLUSION

A total of 20 samples of wheat (*Triticum aestivum*) seeds were collected from different Upazilla of Kustia, Jessore and Jhenaidah. The present study was conducted to isolate and identify the seed borne fungi of collected seed sample of wheat and to determine their effect on germination and seedling vigor. The research work was carried out in the Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 during the period of May to November 2016. The experiment was carried out according to the rules of International Seed Testing Agency (ISTA) with collected seed samples.

In Blotter paper method eight fungal species viz. *Bipolaris sorokiniana Aspergillus flavus, Aspergillus niger, Alternaria tenuis, Fusarium moniliforme, Penicillium* spp., *Curvularia lunata* and *Pyricularia grisea* were observed. The highest incidence of *Penicillium* spp. in variety prodip (6) (15.33%) , *Aspergillus flavus* was recorded in prodip (4) (26.33%), *Aspergillus niger* was recorded in variety prodip (1) (20%), *Bipolaris sorokiniana* was in Shotabdi (6) (17.67%), *Fusarium moniliforme* was recorded in prodip (6) (17.61%) , *Alternaria tenuis* was recorded in variety Shatabdi (10) (14.33%) followed by Sonalika (1) (12.67%), *Curvularia lunata* was recorded in Shatabdi (2) (12.67%) followed by variety Prodip (6) (7.33%) and *Pyricularia grisea* was recorded the highest in variety Shatabdi (2) (5.33%) .

In rolled paper towel method the highest seed germination percentage was recorded in variety Sonalika (1) (81%) . The highest shoot length percentage were recorded in sample no Prodip (4) and Sonalika (4) and Sonalika (3) (14.00%) . The highest root length percentage was recorded in Shatabdi (8) (16.67). The highest weight of seedling was recorded in variety prodip (2) (4.80g). The vigor index of seedling was highest in variety Sonalika (2) (1796) and lowest was in Shatabdi (2) (480).The differences in this parameter at different levels of seed infected by pathogen were significant.

In water agar test tube method, the highest seed germination percentage was (70.00%) recorded in variety Prodip (4).The number of normal seedlings was highest (46.67%) in Sonalika (4). Incase of abnormal seedling, the number of abnormal seedlings was highest in Prodip (4) (50%) .Dead seed percentage was recorded highest in shotabdi (5) (66.67%).

In Deep Freezing method eight fungal species viz. *Bipolaris sorokiniana* , *Aspergillus flavus, Aspergillus niger, Alternaria tenuis, Fusarium moniliforme, Penicillium* spp., *Curvularia lunata* and *Pyricularia grise*a were observed. The highest incidence of *Penicillium* spp. in variety Shotabdi (3) and Shotabdi(9) (9.00%) ,s*Aspergillus flavus* was recorded in Shotabdi (3) (20.00%), *Aspergillus niger* was recorded in Shotabdi (7) (8.00%), *Bipolaris sorokiniana* was in Sonalika (2) (30.00%) , *Fusarium moniliforme* was recorded in variety Shotabdi (8) (21.00%) , *Alternaria tenuis* was recorded in Shotabdi (9) (23.00%), *Curvularia*

lunata was recorded in Sonalika (4) and Shotabdi (9) (14.00%) and *Pyricularia grisea* was recorded in Shotabdi (8) (4.00%) .

Among all the method the maximum germination was counted from Rolled paper towel method. It was 79% which is observed in variety Shotabdi (9).The lowest germination percentage was observed in blotter paper method in variety Shotabdi (2) which was collected from Baganchara, Sharsha, Jessore. Pathogen occurrence were high in Deep freezing blotter method than the blotter paper method.

Seed is a common carrier of plant pathogens. Pathogen free seed is the important input material in agriculture. The present experiment showed that a lot of seed borne pathogens were associated with the collected wheat seed from the particular area. Considering the over-all findings it was revealed that the seed health status of collected wheat seeds is not in a satisfactory level. Farmers are therefore advised to collect the seeds from reliable sources and check the seed health status before sowing in the main field.

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Table 1. Blotter paper method

Sample No.	Gern%	Penicilliu m	Alternari a Sp	Aspergill us flavus	Aspergill us niger	Fusarium	Bipolaris	Curvular ia	grisea Pyricular ia

Sample01	66.40a	4.00 h	11.00 bc	19.67 de	5.000 h	4.00 m	17.33 ab	6.000cde	2.00 d
Sample 02	2.733 l	4.66 gh	5.000 g	26.67 b	14.67 b	12.0def	5.667i	12.67 a	5.33a
Sample03	34.67 c	4.00	5.000 g	34.00a	20.00a	8.00 jk	7.000i	2.333 j	2.00 d
Sample 04	4.667 l	5.00 gh	11.00 bc	18.67 def	11.00f	11.0 efgh	3.000 j	5.330 efg	1.33 f
Sample 05	48.23 b	4.00 h	9.000 de	17.67 ef	9.000 g	7.00 kl	12.67 gh	6.000cde	2.00 d
Sample 06	21.80 fg	6.67 ef	9.000 de	26.33 b	9.000 g	12.3de	13.00 fgh	4.330 hi	1.67 e
Sample 07	27.33 d	5.67 fgh	9.330 cde	20.67 d	13.00 cd	9.00 ij	11.00 h	6.000cde	1.00 g
Sample 08	21.33 fgh	8.00 de	10.00 cd	23.33 c	8.670 g	6.67 kl	13.00 fgh	4.000 i	4.00 b
Sample09	12.33 j	4.00 h	9.000 de	19.00 def	7.670 g	6.00 l	14.67defg	5.670 def	0.33 i
Sample 10	14.00 j	8.67 d	12.67 b	26.67 b	13.33 bc	9.66 hi	14.00 efg	5.000 fgh	3.00 c

Sample 11	8.667 k	9.00 d	12.00 b	18.00 ef	11.33 ef	11.0 efgh	17.00 abc	4.000 i	1.33f
Sample 12	26.03 de	12.0 c	7.000 f	17.67 ef	11.67 def	9.00ij	17.67 a	4.670 ghi	0.670 h
Sample 13	17.10 i	6.00 fg	8.670 de	21.00cd	11.67 def	9.00 ij	13.67 efg	5.670 def	0.000 j
Sample 14	13.33 j	9.33 d	9.670 cd	16.67fg	12.67cde	10.6 fgh	14.33 defg	6.330cd	1.00 g
Sample 15	23.67 ef	11.00 c	12.00 b	13.33 h	8.000 g	11.6 efg	15.00cdef	4.670 ghi	0.670 h
Sample 16	22.00 fg	13.67 b	11.00 bc	10.33 i	13.00 cd	13.3 cd	13.67 efg	5.670 def	0.670 h
Sample 17	20.00 ghi	15.33 a	7.667 ef	13.33 h	13.33 bc	17.6a	16.33 abcd	7.330 b	1.00 g
Sample 18	18.70 hi	11.67 c	11.00 bc	16.67fg	13.67 bc	16.0 b	13.67 efg	5.330 efg	0.670 h
Sample 19	22.00 fg	14.00 ab	12.00 b	14.33 gh	13.33 bc	10.3 ghi	12.67 gh	6.670 bc	1.00 g
Sample 20	24.10 ef	8.670 d	14.33a	18.67 def	13.33 bc	14.0 c	15.33 bcde	4.670 ghi	0.330 i

$LSD_{0.05}$	2.78	1.47	1.53	2.36	1.31	1.39	1.88	0.851	0.195
Level of significance	**	**	**	**	**	**	**	**	**
CV (%)	7.52	10.82	9.48	7.29	6.82	8.13	8.75	9.18	7.87

** = Significant at 1% level of probability

Table 2. Paper rolled method

Sample No.	Shoot length (cm)	Root length (cm)	Weight of seedling (mg)	Germination (%)	Vigor index
Sample01	9.333 cde	13.67 c	3.483 efgh	69.33 efgh	1595. defgh
Sample 02	3.000 f	5.000 g	0.7600 l	60.00 j	480.0 k
Sample03	10.33 bcd	13.50 c	1.603k	70.47 ef	1679.cdef
Sample 04	8.330 e	10.33 e	4.803 b	77.67 abc	1450. hi
Sample 05	10.83 bcd	12.33 d	3.483 efgh	66.00 ghi	1528. fgh
Sample 06	14.00 a	12.67 cd	4.300 bcd	73.67 cde	1964.a
Sample 07	12.00 b	12.33 d	3.907 cdef	71.00 e	1728.bcde
Sample 08	10.33 bcd	12.67 cd	3.253fgh	65.33 hi	1503. ghi
Sample09	9.667 cde	11.00 e	2.090 jk	54.67 k	1130. j
Sample 10	11.33 bc	10.83 e	5.533 a	81.00 a	1796.bc
Sample 11	12.17 b	8.330 f	3.460 efgh	77.00 abcd	1579. efgh
Sample 12	9.170 de	13.00 cd	3.217 gh	66.43 fghi	1473. hi
Sample 13	10.33 bcd	12.67 cd	3.773 defg	71.43 e	1643. cdefg
Sample 14	9.330 cde	15.33 b	4.503 bc	73.20 de	1806. bc
Sample 15	10.33 bcd	14.67 b	2.490 ij	62.67 ij	1567. efgh
Sample 16	14.00 a	12.33 d	3.060 hi	70.00 efg	1844. ab
Sample 17	10.00 cde	10.33 e	3.940 cde	76.10 bcd	1544. fgh
Sample 18	10.67 bcd	16.67 a	3.317 efgh	66.00 ghi	1804. bc
Sample 19	11.00 bcd	11.17 e	4.233 bcd	79.00 ab	1751. bcd
Sample 20	9.333 cde	12.33 d	2.210 jk	60.00 j	1360. i
$LSD_{0.05}$	1.69	0.971	0.592	3.93	143.40
Level of	**	**	**	**	**

significance					
CV (%)	9.93	4.88	10.64	3.43	5.56

** = Significant at 1% level of probability

Table 3. Test tube method

Sample No.	Germination (%)	Seedling (%)	Abnormal seedling (%)	Dead seedling (%)
Sample01	40.00 efg	23.33 e	26.67 f	50.00cd
Sample 02	40.00 efg	10.00 i	36.67c	53.33bc
Sample03	33.33 g	20.00 f	26.67 f	53.33bc
Sample 04	66.67 ab	43.33 b	30.00 e	26.67j
Sample 05	56.67 bc	33.33 c	30.00 e	36.67gh
Sample 06	70.00 a	16.67 g	50.00 a	33.33hi
Sample 07	53.33 cd	23.33 e	36.67c	40.00fg
Sample 08	46.67 cdef	16.67 g	26.67 f	56.67b
Sample09	36.67 fg	3.33 j	30.00 e	66.67a
Sample 10	53.33 cd	26.67 d	36.67c	36.67gh
Sample 11	56.67 c	26.67 d	33.33 d	40.00fg
Sample 12	53.33 cd	10.00 i	36.67c	53.33bc
Sample 13	56.67 bc	23.33 e	40.00 b	36.67gh
Sample 14	40.00 efg	20.00 f	23.33 g	56.67b
Sample 15	73.33 a	46.67 a	23.33 g	30.00ij
Sample 16	40.00 efg	13.33 h	30.00 e	56.67b
Sample 17	50.00 cde	33.33c	20.00 h	46.67de
Sample 18	43.33 defg	13.33 h	30.00 e	56.67b

Sample 19	50.00 cde	16.67 g	40.00 b	43.33ef
Sample 20	53.33 cd	26.67 d	23.33 g	50.00cd
$LSD_{0.05}$	9.82	1.41	1.90	3.71
Level of significance	**	**	**	**
CV (%)	11.75	3.82	3.67	4.87

** = Significant at 1% level of probability

Table 4. Deep freezing method

Sample No.	Penicilliu m	Alternari a	Aspergill us flavus	Aspergill us niger	Fusarium	Bipolaris	Curvulari a	grisea Pyriculari a
Sample01	3.33 ef	12.33 hi	5.333 bcde	4.00 f	12.67 gh	19.67 e	7.00g	2.00 d
Sample 02	3.66 ef	15.00 efg	6.000 abc	4.66de	13.00 fgh	26.67 bcd	5.33 hij	1.67 e
Sample03	6.00c	16.33def	4.333 efg	4.00 f	11.00 h	18.67 ef	4.67 j	1.00 f
Sample 04	2.00 g	13.00 hi	6.000 abc	5.00d	13.00 fgh	17.67 efg	3.67 k	0.67 g
Sample 05	4.00 e	15.00 efg	4.000 fg	4.00 f	14.67efg	26.33 bcd	5.33 hij	1.67 e
Sample 06	7.00 b	15.00 fg	5.670 bcd	2.33 h	14.00efgh	19.00 e	5.66 hi	2.33 c
Sample 07	9.00 a	19.00 c	5.000 cdef	4.33 ef	17.00bcde	18.00 ef	5.33 hij	1.67 e
Sample 08	3.00 f	21.00 b	4.000 fg	6.00 c	17.67bcd	25.00 d	5.66 hi	2.00 d
Sample09	5.00 d	23.00a	4.670 defg	3.00 g	13.67 fgh	28.00 abc	5.00 ij	3.00 b
Sample 10	4.00 e	19.00c	5.670 bcd	5.00d	14.33 efg	24.00 d	6.00 h	1.00 f
Sample 11	3.00 f	16.00 ef	6.333 ab	7.00 b	11.00 h	30.00 a	10.00 d	0.00 h
Sample 12	2.00 g	17.00 de	4.667 defg	6.00c	14.00 efgh	19.00 e	11.00c	1.00 f
Sample 13	5.00 d	15.00 efg	4.667 defg	8.00 a	18.00 bc	16.00 fgh	9.000e	3.00 b
Sample 14	6.00 c	17.00 de	3.667 g	7.00 b	13.00 fgh	29.00 ab	12.00 b	2.00 d
Sample 15	7.00 b	18.00cd	5.333 bcde	6.00 c	15.00 defg	18.00 ef	14.00a	1.00 f

Sample 16	3.00 f	12.00 i	5.667 bcd	4.00 f	16.00cdef	24.00 d	12.00b	0.00 h
Sample 17	2.00 g	14.00 gh	5.333 bcde	2.00 h	11.00 h	26.00 cd	10.00d	1.00 f
Sample 18	7.00 b	17.00 de	5.000 cdef	4.00f	21.00a	15.00 gh	8.000 f	4.00 a
Sample 19	9.00 a	23.00a	6.000 abc	3.00 g	19.00ab	20.00 e	14.00a	3.00 b
Sample 20	5.00 d	21.00 b	7.000 a	2.00 h	12.00 gh	14.00 h	9.000 e	2.00 d
$LSD_{0.05}$	0.672	1.75	0.994	0.574	2.67	2.57	0.738	0.209
Level of significance	**	**	**	**	**	**	**	**
CV (%)	8.50	6.29	11.54	7.60	11.15	7.19	5.50	7.34

** = Significant at 1% level of probability

Appendix I. Blotter paper method

Source of variation	df	Germination %	*Penicillium* sp	*Alternaria* sp	*Aspergillus flavus*	*Aspergillus niger*	*Fusarium moniliforme*	*Bipolaris sorokiniana*	*Curvularia lunata*	*Pyricularia grisea*
Variety	19	629.85**	40.717**	17.523**	93.357**	30.906**	33.540**	43.887**	11.937**	5.171**
Error	40	2.85	0.800	0.867	2.050	0.633	0.717	1.300	0.266	0.014
Total	**59**									

** = Significant at 1% level of probability

Appendix II. Paper rolled towel method

Source of variation	df	Shoot length (cm)	Root length (cm)	Weight of seedling (mg)	Germination (%)	Vigor index
Variety	19	15.244**	18.767**	3.832**	146.156**	303589.048**
Error	40	1.050	0.346	0.129	5.698	7546.656
Total	**59**					

** = Significant at 1% level of probability

Appendix III. Water Agar method

Source of variation	df	Germination (%)	Seedling (%)	Abnormal seedling (%)	Dead seedling (%)
Variety	19	363.519**	358.258**	159.592**	348.34**
Error	40	35.417	0.726	1.339	5.06
Total	**59**				

** = Significant at 1% level of probability

Appendix IV. **Deep freezing method**

Source of variation	df	*Penicillium* spp	*Alternaria* sp	*Aspergillus flavus*	*Aspergillus niger*	*Fusarium moniloformae*	*Bipolaris sorokiniana*	*Curvularia lunata*	*Pyricularia grisea*
Variety	19	14.330***	31.916**	2.221**	8.670**	23.343**	72.488**	31.380**	3.223**
Error	40	0.166	1.133	0.363	0.121	2.633	2.433	0.200	0.016
Total	**59**								

** = Significant at 1% level of probability