STUDY ON THE QUALITY AND HEALTH STATUS OF FARMER SAVED WHEAT SEED IN BANGLADESH

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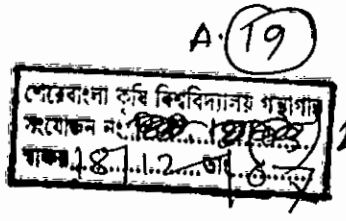
CERTIFICATE

This is to certify that thesis entitled, **"STUDY ON THE QUALITY AND HEALTH STATUS OF FARMER SAVED WHEAT SEED 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 result of a piece of bonafide research work carried out by **Nazmoon Naher Tonu, Roll No. 01517, Registration No. 25483/01517,** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

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



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Dedicated to

My

Beloved Parents

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NAZMOON NAHER TONU

ABSTRACT

Quality and health status of farmer saved wheat seed of cv Kanchan grown in 2004-05 was determined by seed health and quality analysis. One hundred seed samples collected from 100 farmers representing ten upazilas under nine major wheat growing districts of Bangladesh were included for the study. Seed health and quality analysis revealed that moisture content of farmer saved seed ranged between 12.04-13.30%. Six types seed contaminants recorded in farmers' seed were – weed seeds, insects, varietal mixture, seed with husk, other crop seed and inert matter. Farmer saved seed also contained five types of abnormal seeds viz black pointed seed, discoloured seed, undersized seed, shriveled seed and insect damaged seed. On an average, 58.48% 'clean' or 'best' seed was recovered from farmer saved wheat seed by removing the seed contaminants and abnormal seeds through manual seed cleaning. Clean seed had the highest weight, while the undersized and black point Grade 5 seeds were lighter than all other categories of wheat seed. Occurrence of seed contaminants, abnormal seeds and the sorted out clean seed from the farmer saved seed varied depending on the seed sources. Of the 13 fungi encountered in farmers' seeds, five target pathogenic fungi identified, in order of prevalence, were - *Bipolaris sorokiniana, Alternaria tenuis, Curvularia lunata, Fusarium oxysporum* and *Aspergillus flavus*. Incidence of these target fungi in both unclean and clean seeds varied significantly with respect to location of seed collection. All the five target fungal pathogens were more prevalent in farmers saved seed compared to clean seed. 'Clean' or 'best' seed significantly gave higher counts of normal seedlings and lesser percentage of abnormal seedlings and dead seeds over unclean farmers' seed and abnormal seeds like black pointed, undersized, discoloured and shriveled seeds. Similarly clean seed resulted higher germination and seedling vigour index over unclean and abnormal seeds.

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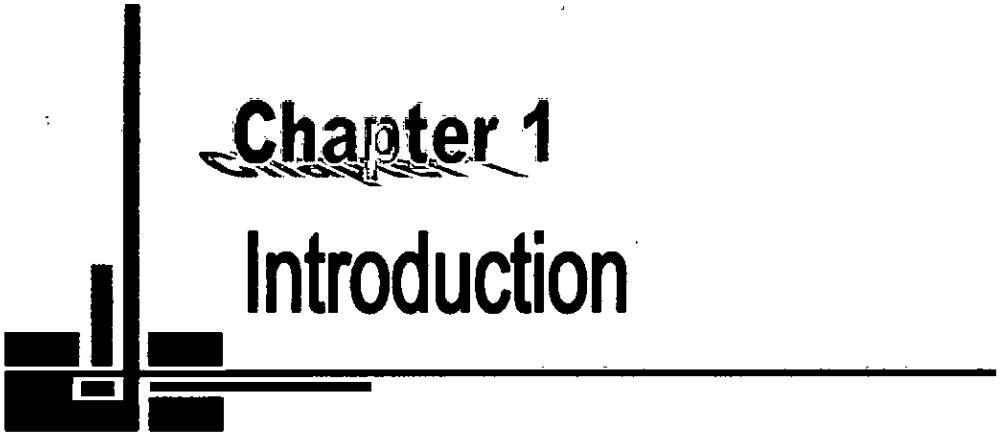


Chapter 1

Introduction

1. INTRODUCTION

Wheat (*Triticum aestivum* L.) is the second most important staple food crop next to rice in Bangladesh. At present, 12.53 million metric tons of wheat produced in 6.25 million ha of land in the country (BBS, 2004). The average yield of wheat is only 1992 kg/ha. in Bangladesh, which is too low as compared to the developing countries of the world like Egypt, Zambia, U.K. Denmark, Ireland and Netherlands producing 6150, 6452, 7778, 7832, 7862, 9110 kg/ha., respectively (FAO, 2003). Thus the yield of wheat in the country is relatively low.

Among the various factors responsible for low yield of the crop, diseases and low seed quality play a vital role. Wheat suffers from as many as 120 different diseases, out of which 42 are seed-borne (Richardson, 1990; Weise, 1991; Mathur and Cunfer, 1993). Coincidentally, seed-borne diseases of wheat are more devastating in nature and have worldwide occurrence. Of all the seed borne diseases, leaf blight (*Bipolaris sorokiniana*), yellow spot (*Bipolaris tritici repentis*), loose smut (*Ustilago tritici*), bunt (*Tilletia* spp.), glume blotch (*Stagonospora nodorum*), head blight (*Fusarium* spp.), black point (*Bipolaris sorokiniana*, *Curvularia lunata, Cladosporium cladosporoides* and *Fusarium* sp.) and germination

failure or seed rot (*Fusarium* spp, *Bipolaris* spp and *Aspergillus* spp.) are of considerable importance. A good number of these diseases outbreak in epidemic form in many wheat growing countries and cause heavy production losses (Bazlur Rashid *et al.* 1983; Weise, 1991; Mathur and Cunfer, 1993; Bazlur Rashid and Fakir, 1998; Fakir, 1998; Bazlur Rashid and Fakir, 2000).

In Bangladesh, 16 different diseases are known to occur on wheat. Of these, 12 are seed borne (Fakir *et al.*, 1990; Fakir, 2001). Among the seed borne diseases, leaf blight caused by *Bipolaris sorokiniana* is the most devastating disease. The disease has been found to break out in epidemic form when there are rains during grain formation and maturation. Leaf blight of wheat is known to cause heavy losses almost every year in the country. Besides leaf blight, other seed borne diseases namely black point (*Bipolaris sorokiniana, Curvularia lunata, Alternaria tenuis, Fusarium* sp.), yellow spot (*Bipolaris tritici repentis*) and loose smut (*Ustilago tritici*) also attack the crop and causes considerable losses to wheat production.

Seed is the most important input for crop production and quality healthy seed is the crying need of the day. Good or quality healthy seed means good crop. This truth is also applicable for wheat seed. In Bangladesh, of

the total wheat seed requirement, only 10% seed produced by BADC, NGOs and seed companies are of good quality to some extent. These seeds are being tested for quality and certified or truthfully labeled. Again, the quality of this seed is judged only by purity and germination. No attention is paid to seed health. The rest 90% wheat seeds are produced by the farmers with little or without care. Traditionally, our farmers grow wheat for consumption purpose. From this produce, they save a portion and keep them as seed. These farmer-saved seeds are never tested for quality. Obviously, these seeds are of poor quality and often infected by pathogens.

Infected wheat seeds fail to germinate or the young seedlings emerging from the infected seeds die resulting germination failure, post emergence damping off and seedling blight. Growing wheat plants in the field from the infected seeds, escaping early infection, may often be infected at the later stages of its growth by the primary seed-borne inoculum grown and multiplied out of the infected dead seeds and seedlings. Later on, these inocula may be transmitted to the healthy growing plants of the same or neighboring plants or even neighboring field resulting disease outbreak, often in epidemic form. Seed-borne pathogens, causing diseases on the growing wheat plants in the field quite often attack spike or spike-lets and subsequently infect the seeds.

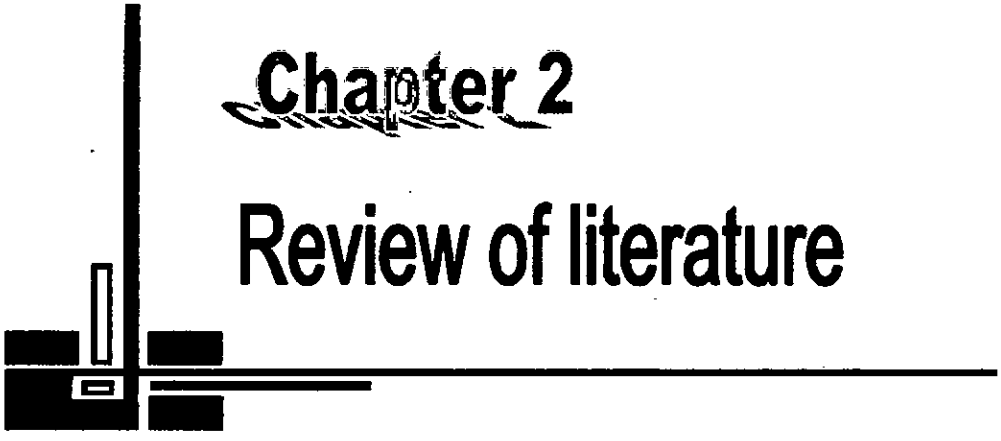
The pathogens present in the infected seeds will remain alive in dormant condition as long as the seed remains viable (Neergaard, 1977). As these pathogens are very small and microscopic in nature their presence in or on the seed cannot be seen from outside with naked eyes. However, sometimes infected wheat seeds may be recognized by observing various kinds of lesions/spots, discoloration, deformation and other abnormalities. Propagules of different pathogens present in dusts, soil particles or infected crop debris (inert matter) may also be mixed with seeds in a given seed lot during the process of harvesting, winnowing and storage. Presence of insects, weed seeds, seeds of other wheat varieties as well as other crop seeds are not also hygienic for the seed lot. Thus, wheat seeds can be infected by seed-borne pathogens or it can carry pathogens as contamination or concomitant contamination. There are also evidences that undersized wheat seeds are poor as regard to seed health quality (Huguelet and Kiesling, 1973; Adlakha and Joshi, 1974; Ram and Joshi, 1979). Such infected seeds and seed lots having contaminants and abnormal seeds signify the poor health and quality of wheat seeds.

In Bangladesh, considerable amount of works have been done on seed-borne fungi, seed health, seed-borne diseases of wheat and their control (Fakir *et.al.*, 1977; Bazlur Rashid, *et al.*, 1992; Bazlur Rashid, 1996;

Bazlur Rashid and Fakir, 1998; Fakir, 1998; Hossain *et al.*, 1998; Bazlur Rashid and Fakir, 2000; Khokon *et al.*,2005). But very limited information is available on the use of quality healthy wheat seeds in the country.

From the foregoing deliberations, it appears that seed-borne diseases are responsible for low yield and production of poor quality wheat seeds in Bangladesh. But the health status of wheat seeds produced by the farmers in the country is not preciously known. But successful wheat cultivation largely depends on the use of quality healthy seeds.

In view of the above facts, the present investigation has been undertaken to determine the quality and health status of wheat seeds produced by the farmers in Bangladesh. The information generated will be useful for further activities to improve farmers'. seed quality, if necessary, for sustaining crop yield.



Chapter 2

Review of literature

2. REVIEW OF LITERATURE

Health of Seed can be affected through direct infection by pathogens without showing any visible symptoms. Often infected seeds may be recognized by observing spots, lesions or different kinds of symptoms produced by the invading pathogens. Propagules of different pathogens present in dusts/soils, crop residues, weed or other crop seeds as contamination or concomitant contamination in a given seed lot also indicate unhealthiness or ill seed health. Thus, like many crop seeds, wheat seeds can be infected by pathogens or a given seed lot of the crop can carry propagules of pathogens as contamination or concomitant contamination. There are evidences that cleaning or removal of infected/abnormal seeds and seed contaminants by manual physical seed sorting can improve the seed health status. An attempt has been made here to collate the relevant literature on the health of wheat seeds affected by pathogens or propagules of pathogens as contamination and benefit of seed cleaning in improvement of seed health by physical seed sorting.

2.1 Health of wheat seeds

Bolley (1913) reported that black pointed wheat grains were usually lighter in weight than normal best grains.

Dastur (1932) reported that the value of crop was appreciably reduced when grains infected with *Helminthosporium sativum* (*Bipolaris sorokiniana*) were used as seed. He found that the discolored seeds germinated poorly and seedlings usually developed blights.

Machacek and Greaney (1938) found that in a large number of cases the kernel weight of discolored wheat grains were greater than the normal grains.

Greaney and Wallace (1943) reported that the *Helminthosporium sativum* reduced the quality of the grain due to the discoloration of kernels through direct lowering of the yield from shrivelling of the grain, through impairment of germination, seedling emergence and yield of the succeeding crop

Hanson and Christensen (1953) reported that the reaction of seed discoloration to germination and plant stand depend on some organism or organisms responsible for discoloration .If *Helntinthosporium* caused it, there may be detrimental effects. They also found an almost similar trend of variation in germination of *Hehninthosporium sativum* infected wheat seeds variety Kubanka collected from different sources. They found the seed germination as 60% and 62% from the seeds having respectively 80% and 74% infection with *H. sativum.* Seed infected with

H. sativum on the other hand, commonly germinated poorly and produced inferior stands with more seedling blight than apparently healthy seeds.

Dharam vir *et al.* (1968) stated that the average incidence of black point disease as based on preliminary survey was from 5-10 percent although considerable variation had been noticed in different samples collected from various sources. Investigations were also conducted to study the effect of this disease in seed germination. The data showed that presence of black point on the seed does not in any way impair seed germination.

Kachalova and Kuzmichev (1969) observed that the incidence of black embryo in the Moscow regions of U.S.S.R. was on winter wheat (1.6%) but higher on spring wheat (10.0% and more). *Alternaria tenuis* (*A. alternata*) attacked winter wheat more frequently; while *Helminthosporium sativum* (*Cochliobolus sativum*) caused more damage to spring wheat. They also observed that besides causing visible symptoms, both the pathogens reduced germination.

Mishra *et al.* (1969) observed that *Drechslera (Bipolaris) sorokiniana* was the most virulent and destructive pathogen in causing grain infection of wheat. Mishra and Singh (1996) observed that among different species

of Drechslera affecting wheat, *D. sorokiniana* was the most virulent and destructive in causing foot rot, leaf blight and grain infection.

Huguelet and Kiesling (1973) reported that *Hilminthosporium sativum* caused light weight shriveled grain.

Adlakha and Joshi (1974) stated that the grains infected with *Helminthosporium sativum* were lighter and smaller than normal ones. In kernels infected by *H. sativum* the discoloration frequently extended to other parts of the grain. Such infected grains were shrivelled and may show fine ridges on the surface.

Ram and Joshi (1979) stated that in glasshouse trials, severe infection of plants from the tillering stage onwards caused yield loss due to the reduction in ear length, number of grains /ear and 1000 grains wt. When the disease occurred only at the flag leaf stage, losses of grain/ear was up to 24%. When infection was confined to the tillering stage, ear length was considerably reduced, affecting the number of grains without shriveling them.

Alam (1980) reported *Drechslera sorokiniana* from freshly harvested wheat seeds grown in Bangladesh. The fungus was pathogenic, causing germination reduction and leaf blight of wheat.

Ali (1981) detected *Fusarium graminearum* and *F. oxysporum* frequently in wheat seeds grown in Bangladesh. He found *Alternaria tenuis, Drechslera sorokiniana,* and *Fusarium* spp pathogenic to germinating seeds and seedlings of wheat.

Rana and Gupta (1982) investigated the incidence of black point in different varieties of wheat and the effect of the disease on germination and root, shoot growth of wheat seedlings. Incidence of black point was 3 to 4%, which was caused by *Helminthosporium sativum.* Black point infections greatly affected not only seed germination but also the shoot growth of the seedlings, the effect being very prominent on root growth.

Sinha and Thapliyal (1984) stated Triticale seeds, infected with black point when planted on PDA, yielded *Helminthosporium sativum* (57%) and *Alternaria tenuis* (35%), the remaining 8% showed some unidentified fungi. It was concluded that *H. sativum* and *A. tenuis* are the two main organisms associated with black pointed grains.

Sinha *et al.* (1984) found maximum reduction (38%) in germination of wheat seed infected with black point pathogen.

Chaudhary *et al.* (1984) studied the effect of black point disease on germination of the grain 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 have been 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 in some cases.

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 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. cause whitish to pinkish discolouration of such grains.

Khanum *et al.* (1987) stated that black point is responsible for the failure of germination of a high percentage of grains in the field. Visual observations indicated that natural infection of grains of the cultivars Lyp-73, Pari-73 and Pak-81, were 50%, 35% and 15%, respectively. The germination of healthy grains was 55-96.5% and that of diseased grains 34.5-71.0%.

Fakir *et al.* (1989) observed the effect of five black point fungi on germinating seeds and emerged seedlings when naturally infected wheat

seeds were incubated by blotter method and seedlings symptoms test, respectively. Among the test fungi, *Drechslera* and *Fusarium* were found pathogenic, capable of causing germination failure/seed rot and/or infection to the emerged seedlings in both the methods. The former caused more seedling infection.

Kalaknnavar *et al.* (1989) graded wheat seeds into 4 size of (a) 2.78-2.38; (b) 2.38-2.78: (c) 1.98-2.38 (d) 1.58-1.98 mm. Percent germination in seeds of (a) and (b) grade was similar and higher than in smaller seeds of (c) and (d) grades. Percent germination was higher in the heavy seeds than in light seeds. Root length and seedling dry weight were found to decrease with the decrease in seed size. Heavy seeds were superior to light seeds in seedling DW and vigour index.

Zhang *et al.* (1990) reported that the grain weight of wheat affected by black point disease in Shaanxi Province, China was 1.95-13.5% lower than uninfected grains. The level of infection depends on environmental factors, cultivars and locations. The predominant pathogen was identified as *B. sorokiniana* in 21 to 54.4% of grains and its pathogenicity was confirmed. *Alternaria tenuis* was present in 8.8 to 35.7% of grains, but was not an important pathogen of black point disease unless associated with other fungi.

Bazlur Rushid *et al.* (1992) observed little variation in the prevalence of seed borne *B. sorokiniana* on wheat with respect to cultivar but marked variation was found with respect to geographic location or seed sources.

Talukder and Fakir (1993) reported that the development of black point of wheat and occurrence of black point fungi, *Drechslera sorokiniana* being predominant in developing grains were not proportional indicating that the fungi might not be the absolute cause of black point disease during grain development.

Agarwal *et al.* (1993) reported that wheat grains with dark brown to black discoloration, generally restricted to the area around the embryo (typical black-point symptoms), showed 100% infection by *A. alternata.*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 creamy white or pinkish colour, mostly shrivelled and lighter in weight were infected by *Fusarium graminearum* [*Gibberella zeae*].

In Bangladesh among the black point fungi of wheat *D. sorokiniana* was found pathogenic and capable of causing germination failure/seed rot and disease to the emerged seedlings (Anonymous, 1995).

Bazlur Rashid (1997) reported highly significant effect of seed borne infection by *Bipolaris sorokiniana* on the germination of seeds of wheat cvs. Kanchan and Sonalika. At the maximum seed borne infection level (90%) both the cultivars Kanchan and Sonalika resulted the minimum germination of 30.25% and 26.50%, respectively. Relationship between the levels of seed borne infection and 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 seed borne infection in both the cultivars. The maximum germination reduction was 71.50% and 18.00% in cv. Sonalika and cv. Kanchan, respectively.

Hossain (1997) studied the status of leaf blight of wheat in Bangladesh. He reported that the artificial inoculation of wheat plant with *B. sorokiniana* at the flag leaf stage reduced the number of grain per ear and 1000-grain weight by 7-100% and 12-100% respectively compared to control.

Fakir (1998a) observed that black point disease surveyed in Balaka, Kanchan and Sonalika varieties of wheat seed in breeders, foundation and certified tiers collected in 1985-86 growing season from nine different wheat growing areas in Bangladesh varied in prevalence from 4.08 to 13.80% with respect to tier. Five different black point fungi detected in

black point affected seeds in the country were: *Alternaria tenuis. Cladosporium cladosporioides, Curvularia lunata, Drechslera sorokiniana* and *Fusarium* sp. Of these, *D. sorokiniana* followed by *A. tenuis* appeared to be the most prevalent black point fungi. Among the black point fungi, *D. sorokiniana* and *Fusarium* sp. were found pathogenic, capable of causing germination failure /seed rot and disease to emerged seedling. No significant effect of sowing up to 12% black point infected seeds of Kanchan on seed yield and development of black point in the field was observed. Seeds with 0 and 6% levels of black point infection had no effect on germination, but 12% significantly reduced germination.

Rahman and Islam (1998) studied that the effect of black point seeds of wheat on its qualitative characters such as weight of I000grain, total crude protein, total crude fiber, total ash, dry matter, and shoot vigour in respect of germination and root growth into five different grades (Grade 0, I, II, III, and IV) on the basis of level of black point infection. All these qualitative parameters, except total crude proteins decreased significantly with the increase of black point infection. The decrease was more pronounced in grade II and grade VI infected seeds. Germination percentage decreased sharply with the increased severity of the disease,

while shoot and root growth also decreased as the grade of infection increased.

Khare and Satpute (1999) reported that germination and seedling vigour were significantly influenced by seed size but not by days to maturity. Small and medium seeded genotypes had better germination and mobilization efficiency than the bold seeded genotypes, which had higher vigour index.

Tomer and Maguire (1999) reported that healthy seeds with high seedling vigour, germinated more synchronously than low vigoured unhealthy seeds.

Hossain (2000) observed that germination of wheat seed in blotter and seedling emergence in pot culture were significantly decreased with the increase in number of black pointed seeds. He also found sharp decrease in germination as the severity of black point infection increased.

2.2. Effect of seed cleaning

Literature available on the effect of seed cleaning on wheat is limited. The available literature on the subject on wheat has also been compiled here along with other crops.

Sultana *el al.*(1992) reported that only 2.0% to 10% and 5% to 12% good/healthy seeds of jute were found in the infected pods of diseased and healthy plants, respectively. Apparently healthy/good seeds of infected pods of diseased and healthy plant showed respectively 66.0% and 65.0% germination with 23.0% and 20.0% infected seeds indicating that seeds from diseased pods showed germination below standard of National Seed Board (NSB). Seeds from healthy pods of healthy and diseased plants gave 98.0% and 95.0% germination, respectively with no seed borne pathogens.

Hossain and Doullah (1998) observed that seed cleaning (use of apparently healthy seed) and washing of farmers' seed reduced the seedling diseases upto 53.87% over the uncleaned farmers' rice seed. They further reported that seed cleaning (apparently healthy seeds) of wheat resulted good plant stand than use of uncleaned seeds (Diseased seeds). Even cleaning of farmer's seed and BADC seeds resulted respectively 83.1% and 92.88% higher plant population in the field.

Chowdhury (1999) observed that cleaning seeds of mustard significantly increased the percent seed germination. The percent seed germination of cleaned seeds and uncleaned seeds were 93.04 and 62.31 respectively collected from healthy pods (siliqua). On the other hand, percent seed germination of cleaned seeds and uncleaned seeds

(Diseased seeds) were 88.96 and 54.65 respectively collected from the uncleaned seeds.

Fakir and Islam (1990) reported that the percent germination of Jute seed was found to be negatively correlated with prevalence of seed borne fungal flora. Prevalence of seed borne fungal flora were higher in farmers' uncleaned seed which resulted poor germination.

In a study to evaluate the cleaning methods to improve the quality of farmer saved rice seed, Rahman *et al.* (2000) found reduced incidence of fungi in cleaned seeds compeared to farmers original saved seeds. Seed cleaning also resulted significant increase in seedling growth and normal seedling and decrease in diseased seedling over the original farmer saved seed.

Hasan *et al.* (2001) reported that prevalence of seed borne pathogenic fungi was lower in manually clean rice seed than farmer seeds. Germination test showed that manual seed cleaning and washing with water increased germination and vigour index by 8.33% and 31.01%, respectively. They also noted significantly higher (12.12%) seedling emergence in seed bed when farmer's seeds were manually clean and washed. Number of diseased seedlings was significantly reduced in seed bed when clean and washed farmers seeds were used. Highest

healthy seedlings (76.62%) was recorded where farmers cleaned and washed seeds were sown.

In studying the quality of rice seed (BR11), Rahman (2000) found that farmers' saved seed had admixture of other varieties, unfilled grains, insect damaged seeds to the extent of 5.9, 9.3 and 7.9% respectively, while spotted and discolored seed ranged from 26.9 to 95.8% germination on test of apparently healthy or clean seeds obtained by visual seed sorting, conducted in soil revealed that seed germination increased by 7-45% over unclean farmers' seed. Visually sorted clean seed yielded significantly reduced percentage of pathogenic fungi, diseased seedlings and dead seeds, but produced increased number of normal seedlings.

Meah (2002) separated apparently healthy seeds from original farmers' seeds and seed from healthy fruits of eggplant. Apparently healthy clean seeds and seeds from healthy fruits showed higher speed and rate of germination and did not have infection of *Phomopsis vexans*.

On seed health analysis of eggplant, Hawlader (2003) observed that farmers' original seed, sorted out apparently healthy and diseased seed gave 20-91%, 26-100% and 17-85% germination, respectively. Dead and rotten seeds were also lower in apparently healthy seed than that of

farmers' original and diseased seeds. The highest *Phomopsis vexans* infection was found in farmers' original diseased seed. The lowest seed-borne infection of the pathogen was encountered in apparently healthy seed. In net house experiment, highest seed germination (91%), no damping off and seedling blight was observed from apparently healthy seeds sown.

Siddique (2003) observed that apparently healthy or clean wheat seeds obtained by bamboo sieving showed better performance in respect of germination, seedling vigour and seed health compared to seeds that dropped under the sieve. Sieved apparently healthy seed had reduced infection of pathogens- *B. sorokiniana, Alternaria tenuis, Aspergillus* spp. and *Fusarium* spp.

Kabir *et al.*(2005) observed that Seed Cleaning by physical seed sorting, an eco-friendly seed treatment practice reduced significantly the population of seed-borne pathogenic fungi in untreated original farmer saved rice (cv. BR 28) seed. They also obtained higher percentage of germination and normal seedlings in Vitavax treated seed followed by washed cleaned seed over untreated original farmer saved seed.

Islam (2005) categorized egg plant seeds into three groups viz. farmers' seed, apparently health seed and diseased seed by manual seed sorting.

He observed least infection of *Phomopsis vexans* and highest germination in apparently healthy seed, followed by farmers' seed and diseased seed. He showed that seed selection had significant influence on the incidence of seed-borne infection of *P. vexans* and use of apparently healthy seed was effective in controlling damping off, tip over and seedling blight of egg plant caused by the pathogen in the nursery bed in the net house.

Khokon *et al.* (2005) reported that analysis of inert matter sorted out from wheat seeds (Cultivar-kanchan) yielded *Bipolaris sorokiniana*, *Fusarium moniliforme, Aspergillus* spp and *Penicillium* sp. The original seed samples (from where the inert matter was sorted out) also yielded the similar fungal pathogens, with higher frequencies. They also detected *Bipolaris oryzae, Alternaria padwichii, Fusarium* spp, *Aspergillus* spp. and *Penicillium* sp. in inert matter separated out of the rice seed samples of cultivars BR-3, BR-14 & BRRI Dhan-29. The original rice samples also yielded the same fungi, in different frequencies.

Chapter 3

Materials and Methods

3. MATERIALS AND METHODS

3.1. Experimental Sites

The seed quality analysis and the laboratory experiments were conducted in the Departments of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka, and Seed Pathology Centre (SPC), Bangladesh Agricultural University (BAU), Mymensingh.

3.2. Experimental Period

The experiments were conducted from July 2005 to June 2006.

3.3. Locations of Seed Collection

Ten locations under 10 upazilas representing nine major wheat growing districts of Bangladesh were selected as sources of seed collection for studying the health and quality status of farmer saved wheat seeds (Fig1). The ten selected upazilas/locations are as follows:

1. Atghoria, Pabna
2. Dinajpur, Sadar
3. Faridpur, Sadar
4. Ishurdi, Pabna
5. Jhikargasa, Jessore
6. Koshba, Brahmanbaria
7. Mymensingh, Sadar
8. Rajbari, Sadar
9. Sherpur, Bogra
10. Ulipur, Kurigram

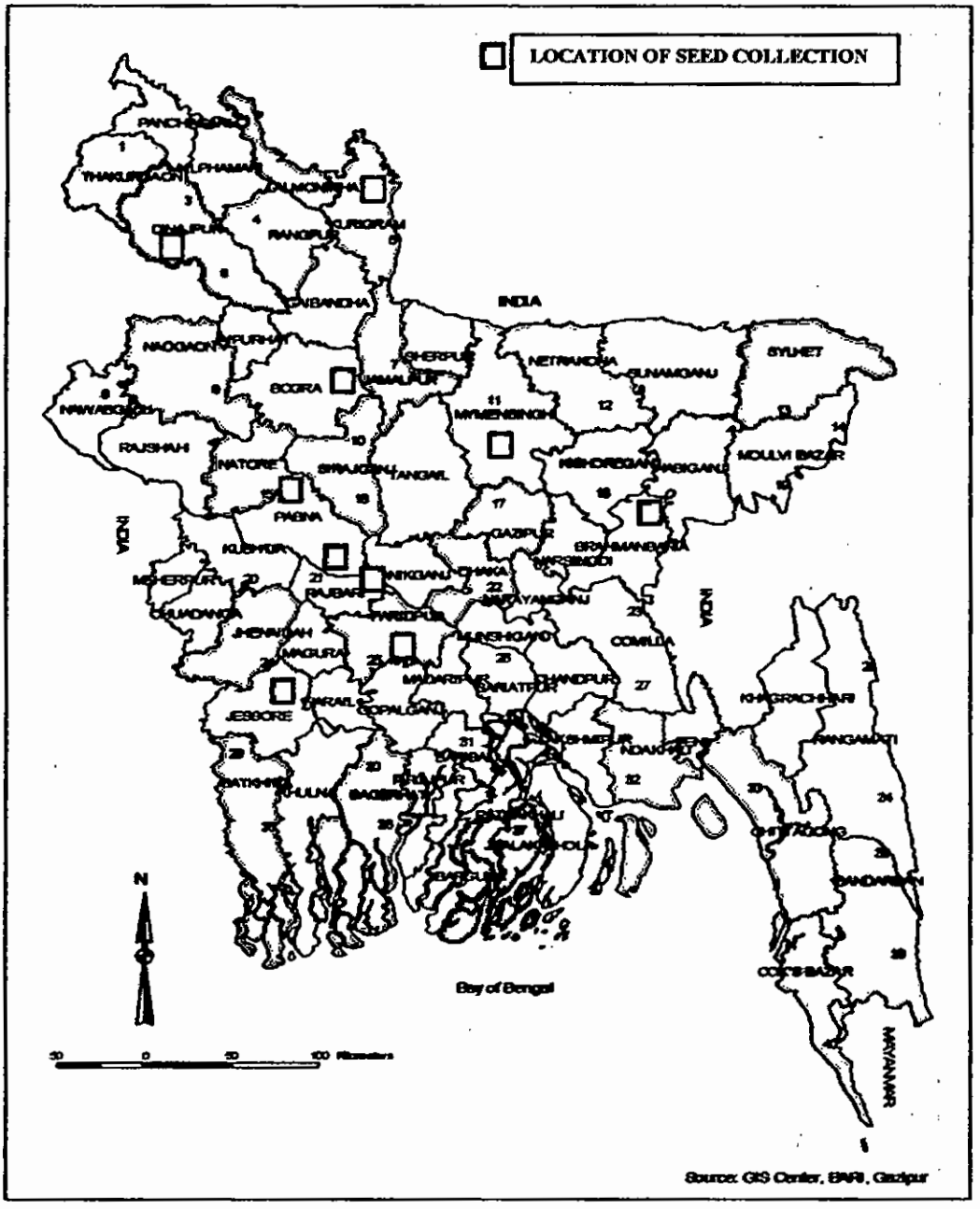


Fig. 1. Map of Bangladesh showing the locations of seed collection

3.4. Cultivar Used

A popular and widely cultivated wheat cultivar Kanchan was used for the study.

3.5. Collection of Seed Samples

One hundred wheat seed samples representing 10 farmers under 10 Upazilas belonging to nine different districts were included for the present study. Ten farmers were selected randomly from one village under each Upazila (Appendix 1). 500g wheat seeds were obtained from each farmer. Seed samples were collected from farmer's seed store by removing top 15cm seed during July - August 2005 following the International Rules for Seed Testing (ISTA, 2001). The seed samples, thus collected, were brought to the Department of Plant Pathology, Sher-e-Bangla Agricultural University (SAU), Sher-e-Bangla Nagar, Dhaka.

3.6. Preservation of Seed Samples

The collected seed samples were kept in cloth bag and stored immediately in a well-ventilated room of the Department of Plant Pathology, SAU, under ambient condition for a short period. The seed samples were then registered. After registration the samples were preserved in a refrigerator in the laboratory.

3.7. Seed Quality and Health Analysis

3.7.1. Determination of moisture content

Moisture content of the seed samples were determined prior to temporary storage by a digital electric moisture meter and the results were expressed in percentage on wet weight basis.

3.7.2. Sorting of 'Best' or apparently healthy seeds

Cleaning of seed by manual seed sorting was done on a clean laboratory table in order to separate out the 'Best' (Apparently healthy) seeds from the original farmer saved seeds. In doing so, seed contaminants and abnormal seeds (seed conditions) present in a 250g working sample of farmer saved seeds were looked for and separated out from each working sample. Seed contaminants separated and recorded from each sample were as follows:

- Weed seeds
- Insects
- Varietal mixture
- Seed with husk
- Other crop seeds
- Inert matter

Abnormal seeds sorted out and recorded were as follows:

- Undersized seeds
- Black pointed seeds
- Discoloured seeds
- Shriveled seeds
- Insect damaged seeds

Apparently healthy seeds obtained from a seed sample by cleaning or removing seed contaminants and abnormal seeds is considered as 'Clean seed'. Inert matter were included, crop residues (broken seeds, husk, awns, etc.), stones, soil and sand particles etc.. Undersized seeds were considered those seeds which had diameter below 2.25mm. Insect damaged seeds were identified by observing the circular hole made by the invading insects. The separation of contaminants and abnormal looking seeds were done with unaided eyes. Occurrence of weed seeds and insects were counted species-wise in number. The rest of the seed contaminants and abnormal seed conditions were recorded and expressed in percentage of seed weight.

3.7.3. Grading of black pointed seeds

The grading of black pointed seeds (Plate 1) was done following the 0 - 5 rating scale of CIMMYT (Gilchrist, 1985). The rating scale is as follows:

0 = free from infection

1 = only embryo blackish

2 = embryo and its adjacent area slightly infected

3 = embryo and less than $^1/_4$th of the grain is discolored

4 = embryo and $^1/_2$ of the grains are infected and

5 = Grains are shriveled, almost completely discoloured or more than $^1/_2$ of the grain discoloured

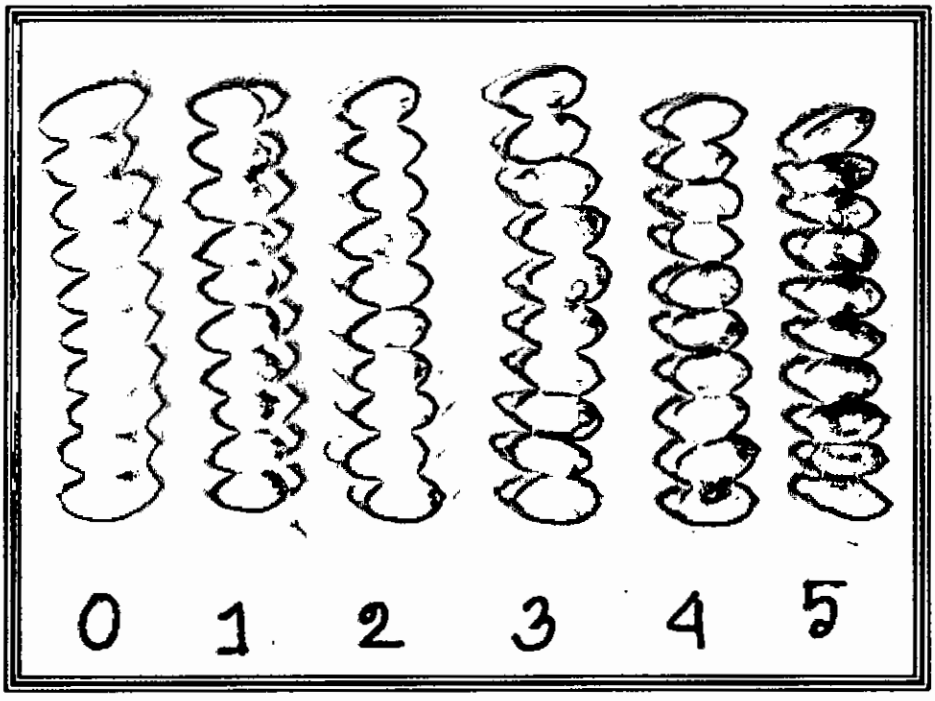


Plate 1. Black pointed wheat seeds of different grades (0-5)

3.7.4 Determination of seed weight

Weight of 1000 seeds of different seed categories of wheat *viz.* Farmer saved seed, Black pointed seed, Undersized seed, Shriveled seed and Best seed was measured by a digital balance. Similarly, 1000 seed weight of the six Black point Grades (0-5) were also measured. The results were expressed in gram (g).

3.7. 5. Detection of seed- borne fungi

Two hundred seeds randomly taken from each working sample were used for detection of seed-borne fungi prevalent in the test seed samples. The fungi were detected by the Blotter method (ISTA, 2001). In this method, three layers of filter paper (Whatman No.1) soaked in water and were placed at the bottom of a 9 cm dia. plastic petridish. Twenty five seeds were then placed on the moistened filter paper in each petridish. The petridishes with seeds were incubated at 20 ± 2°C under 12/12 hours alternating cycles of Near Ultraviolet (NUV) light and darkness in the incubation room of the Seed Health Laboratory, Department of Plant Pathology, SAU for seven days. After incubation, the seeds were examined for the presence of seed borne fungal infections. The fungi were recorded by observing their growth characters on the incubated seeds under stereomicroscope at 25X magnification. The fungi thus recorded were identified following the keys of Ellis (1960), Malone and

Muskett (1964), Raper and Funnel (1965), Booth (1971) and Mathur and Kongsdal (2003). In doubtful cases, slides were prepared and observed under compound microscope for confirmation of identification of the fungi. Fungi thus recorded and identified were expressed in percentage. Of all the fungi detected in wheat seed samples analysed, five fungi *viz. Bipolaris sorokiniana, Alternaria tenuis, Curvularia lunata, Fusaruim oxysporum* and *Aspergillus flavus* were established seed borne pathogens of wheat. As such, they were considered as the target pathogenic fungi for the study

3.7.6. Determination of germination

Germination of original farmer saved seed, best or 'clean seed', black pointed seed, undersized seed and shrivelled seed were determined in clean sand. Plastic trays were used for this purpose. Four hundred seeds in four replicates taken randomly from each seed lot mentioned above were used for germination test. Hundred seeds were sown in each tray and the trays were then kept on the Laboratory table at room temperature for 14 days. Germination was recorded up to 14 days at an interval of 2 days. Normal seedlings, abnormal seedlings and dead seeds were recorded separately following the International Rules for Seed Testing (ISTA, 2001) with some modifications. The modifications included-seedlings with slight defects and seedlings with secondary infection were

placed under the abnormal seedling category, instead of normal seedlings. The results were expressed in percentages.

Normal seedlings were categorized by the following criteria:

i) Intact seedling with all essential structures well developed, complete in proportion and healthy

Abnormal seedlings were categorized using following criteria:

i) Seminal roots missing/stunted or broken and decayed due to primary infection

ii) Coleoptiles missing/split and deformed or bent over

iii) Shoot system (the mesocotyl if developed) broken/decayed

iv) Leaf missing/extending less than halfway up the coleoptile, shredded or deformed

v) Seedling as a whole deformed spindly, discoloured or decayed as a result of primary infection

vi) Seedlings with slight defects and seedlings with secondary infection

vii) Blackened dead or decayed seed.

The number of seeds that produced normal seedlings were counted and the percentage calculated over the number of seeds placed for the test. Similarly, the data on abnormal seedlings and dead or decayed seeds were recorded.

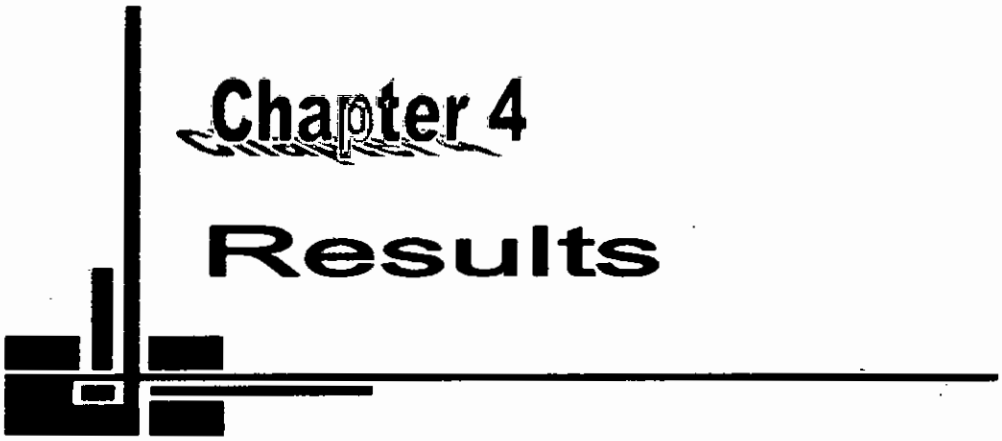
3.7.7. Determination of seedling vigour

Seedling vigour was determined by recording the speed of germination, which was done by taking data on seed germination at 2 days interval. For determination of seedling vigour after two weeks of germination, 25 seedlings were randomly selected and the individual root and shoot length for the selected seed categories viz.- farmers saved seed, black pointed seed, undersized seed, shriveled seed and best seed were measured. Shoot and root were separated from the seedlings and length of shoot was measured from the base of the stem upto the growing point of the youngest leaf. Similarly, length of the root was measured from the starting point of root to the largest available lateral root apex. Seedling vigour was determined by the following formula given by Baki and Anderson (1972).

Vigour index = (Mean of root length + Mean of shoot length) x seed germination (%)

3.8. Statistical Analysis

The recorded data were analysed using MSTAT-C computer package program. The mean differences for efficiency of the treatments were judged by Least Significant Difference (LSD).



Chapter 4

Results

4. RESULTS

4. 1. Moisture Content

The average moisture content of farmers saved wheat seed obtained from 10 different locations of Bangladesh varied significantly from 12.04 – 13.30% (Fig. 2). The maximum moisture content (13.30%) was found in seeds collected from Sherpur, followed by Atghoria (13.20%) and Ishurdi (13.19%), whereas the minimum moisture content (12.04%) was recorded at Mymensingh followed by Dinajpur (12.09%) and Ulipur (12.17%).

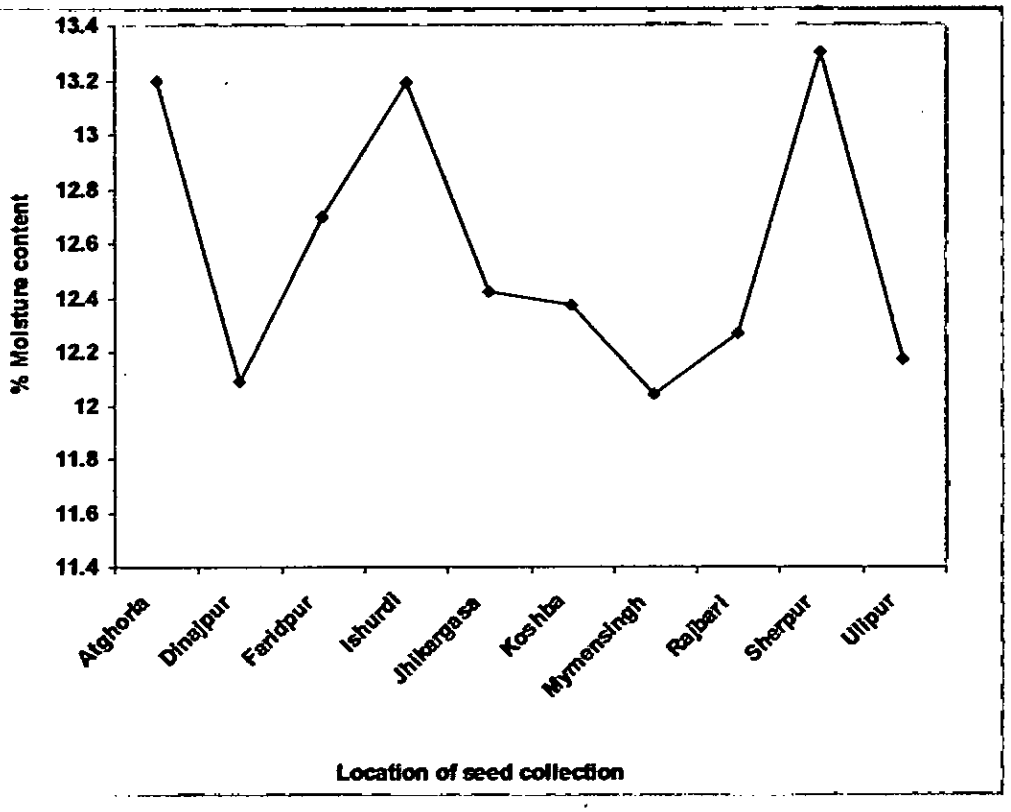


Fig. 2. Moisture content of farmer saved wheat seed collected from 10 different locations of Bangladesh

4. 2. Seed contaminants

Seed contaminants and its frequency of occurrence in the farmers saved seeds are presented in Table 1 and Plates 2 – 7. Six different types of seed contaminants *viz.*, weed seed (Plate 2), insects (Plate 3), varietal mixture (Plate 4), seed with husk (Plate 5), other crop seeds (Plate 6) and inert matter (Plate 7), were recorded in the farmers saved wheat seeds. The seed contaminants varied significantly with respect to location of seed collection (Table 1).

Six different species of weed seeds (*Brassica kaber, Chenopodium album, Cyperus* sp., *Echinochloa crusgalli, Solanum nigrum, Polygonum hydropiper* and *Vicia sativa*) were found as seed contaminants in farmer saved seed. Weed seeds were recorded in farmer saved seeds at all the locations. The highest number (3.00) of weed seeds was observed at Jhikargasa followed by Mymensingh (2.70) and Faridpur (2.60) and Dinajpur (2.50), while the lowest (1.30) was encountered at Ulipur (Table 1).

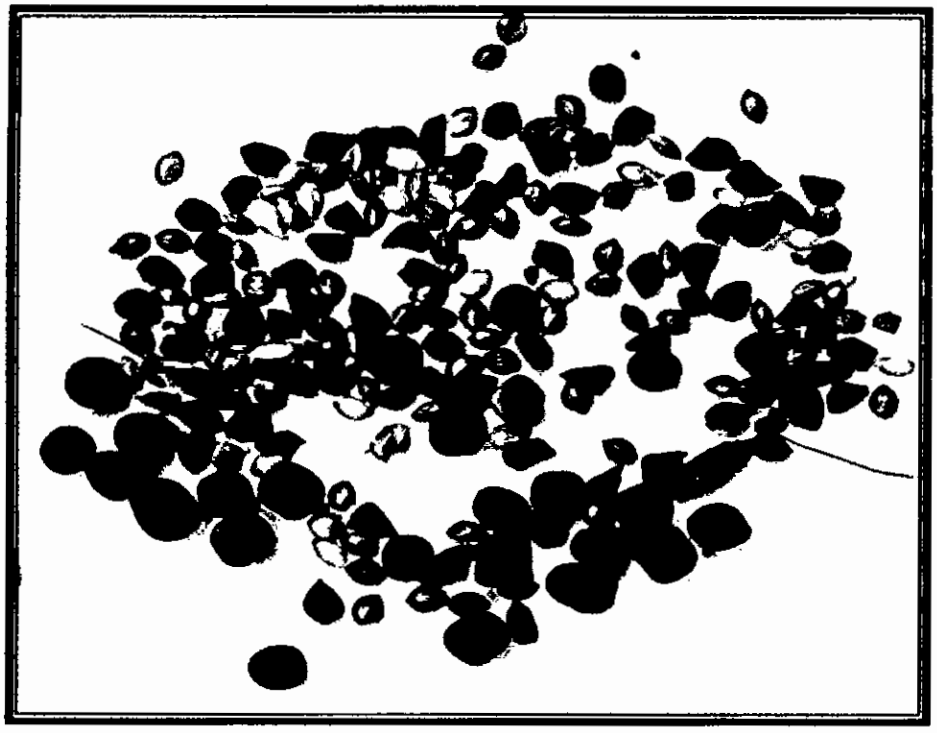


Plate 2. Weed seed sorted out form farmers' saved wheat seeds

A

B

C

Plate 3. Insects sorted out from farmer saved wheat seeds

A. Weevil, B. Rice moth, C. Red flour beetle

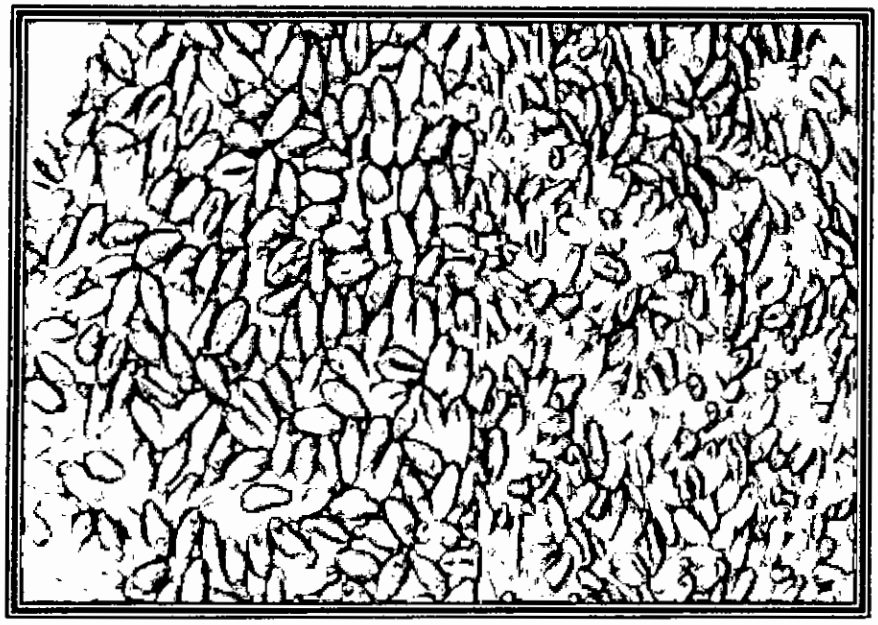


Plate 4. Varietal mixture sorted out from farmers' saved wheat seed.

Plate 5. Seed with husk sorted out from farmers' saved wheat seed.

Plate 6. Other crop seeds sorted out from farmers' saved wheat seed.

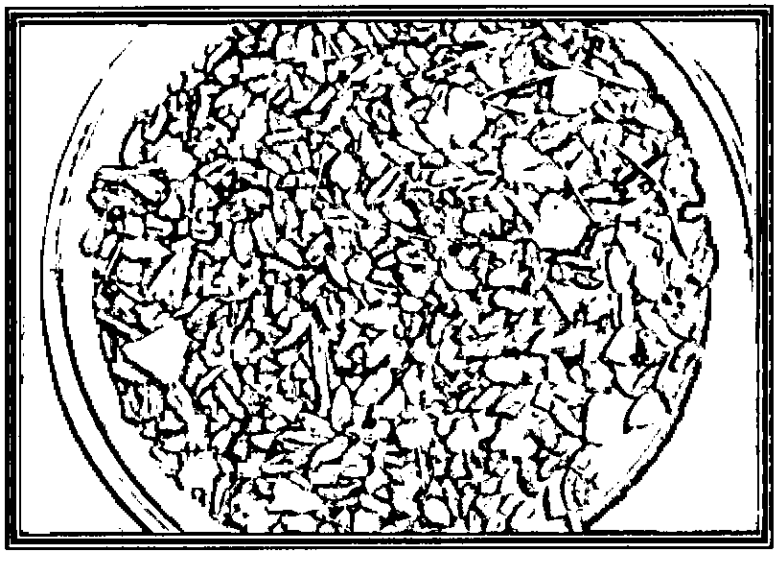


Plate 7. Inert matter sorted out from farmers' saved wheat seed.

Three species of insects *viz*, red flour beetle (*Tribolium casteneum*), rice moth (*Corcyra cephalonica*) and weevil (*Sitophillus oryza*) were observed in farmer's saved seed during seed quality analysis. The three insects were recorded in seeds collected at all the locations. Maximum number (2.80) of insects was found at Dinajpur followed by Faridpur and Mymensingh (2.60), while the minimum number (0.70) was recorded at Rajbari (Table 1).

The prevalence of varietal mixture in farmer saved seed varied significantly from 0.06 – 3.90% with respect to source of seed collection. The average mixture of other varieties with the collected seed samples was the highest at Ulipur (3.90%) followed by Ishurdi (3.89%) and Dinajpur (3.86%), while the lowest (0.04%) was recorded at Mymensingh (Table 1).

The occurrence of seed with husk, other crop seed and inert matter as seed contaminants in farmers' seed was found in low quality at all the locations with some exceptions. Seed with husk was present at all the locations of seed collection. It varied from 0.09 – 0.35% depending on the seed sources. Other crop seed also occurred at all the locations ranging from 0.05 – 1.68%. Maximum association of other crop seeds were found at Ishurdi (1.68%) and the minimum was encountered at Koshba (0.05%). The percentage of inert matter ranged from 0.24 (Mymensingh) to 2.65% (Jhikargasa) (Table 1).

Table 1. Seed contaminants recorded in farmers' saved wheat seed collected from different locations of Bangladesh

Location	Seed contaminants					
	Weed seed (No.)	Insect (No.)	Varietal mixture (%)	Seed with husk (%)	Other crop seed (%)	Inert matter (%)
Atghoria	1.80 b	1.60 b	2.56 c	0.35 a	0.22 ef	0.60 d
Dinajpur	2.50 a	2.80 a	3.86 a	0.16 bcd	0.29 de	0.38 ef
Faridpur	2.60 a	2.60 a	1.79 d	0.17 bcd	0.71 c	0.96 c
Ishurdi	1.70 b	1.70 b	3.89 a	0.09 d	1.68 a	0.45 e
Jhikargasa	3.00 a	1.90 b	0.12 e	0.16 bcd	0.21 ef	2.65 a
Koshba	1.70 b	1.60 b	3.23 b	0.25 abc	0.05 g	0.44 e
Mymensingh	2.70 a	2.60 a	0.06 e	0.18 bcd	0.07 g	0.24 f
Rajbari	1.60 b	0.70 c	2.36 c	0.11 cd	0.12 fg	1.19 b
Sherpur	1.70 b	1.60 b	1.61 d	0.29 ab	0.91 b	0.36 ef
Ulipur	1.30 b	0.80 c	3.90 a	0.12 cd	0.34 d	0.90 c
LSD ($P \geq 0.01$)	0.63	0.57	0.35	0.14	0.12	0.15
CV (%)	25.84	26.92	12.72	61.59	20.33	15.34

** = 1% level of significance

Analysis was performed after square root transformation of the % data.

4. 3. Abnormal seeds

Data on five different types of abnormal seeds recorded in farmer saved wheat seed collected from ten selected locations are presented in Table 2 and Plates 8-12. Five types of abnormal seeds encountered were undersized seed (Plate 8) black pointed seed (Plate 9), discoloured seed (Plate 10), shriveled seed (Plate 11) and insect damaged seed (Plate 12). The prevalence of abnormal seeds varied significantly depending on the types of abnormal seeds and location of seed collection (Table 2). Among the different types of abnormal seeds, undersized seed was the predominant, followed by black pointed seed.

The highest occurrence of undersized seed was recorded in Sherpur (33.01%) followed by Mymensingh (31.61%) and Ulipur (29.55%); while the lowest record of undersized seed was found in Jhikargasa (16.52%). Prevalence of black point in farmers' saved seed varied significantly from 3.38% – 28.53% depending on the location of seed collection. The highest count of black point was observed at Sherpur (28.53%), followed by Jhikargasa (25.12%) and Dinajpur (10.71%); while the lowest was encountered at Faridpur (3.38%).

Plate 8. Undersized seed sorted out from farmers' saved wheat seed.

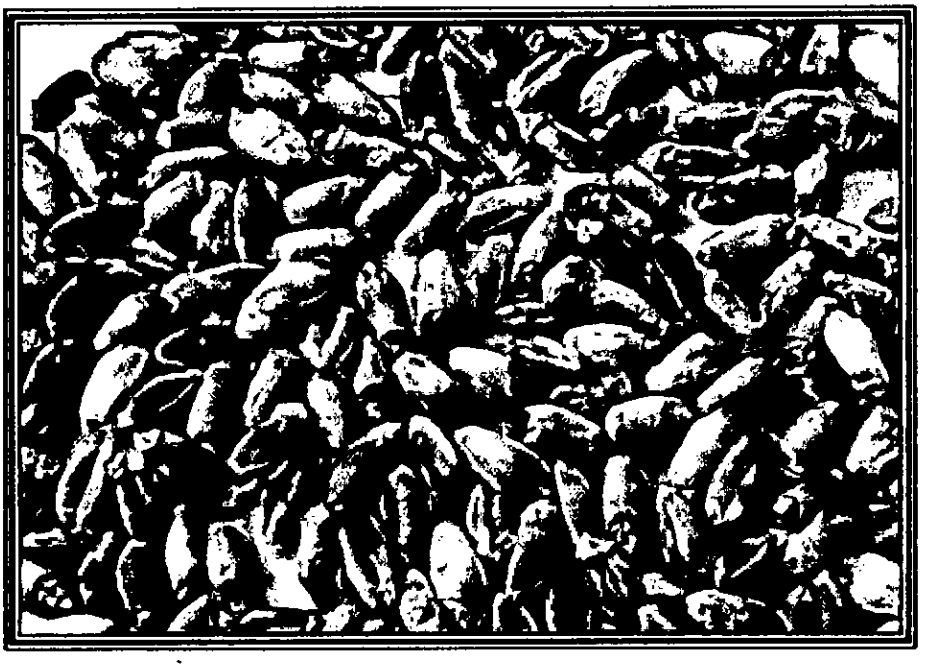


Plate 9. Black pointed seed sorted out from farmers' saved wheat seed.

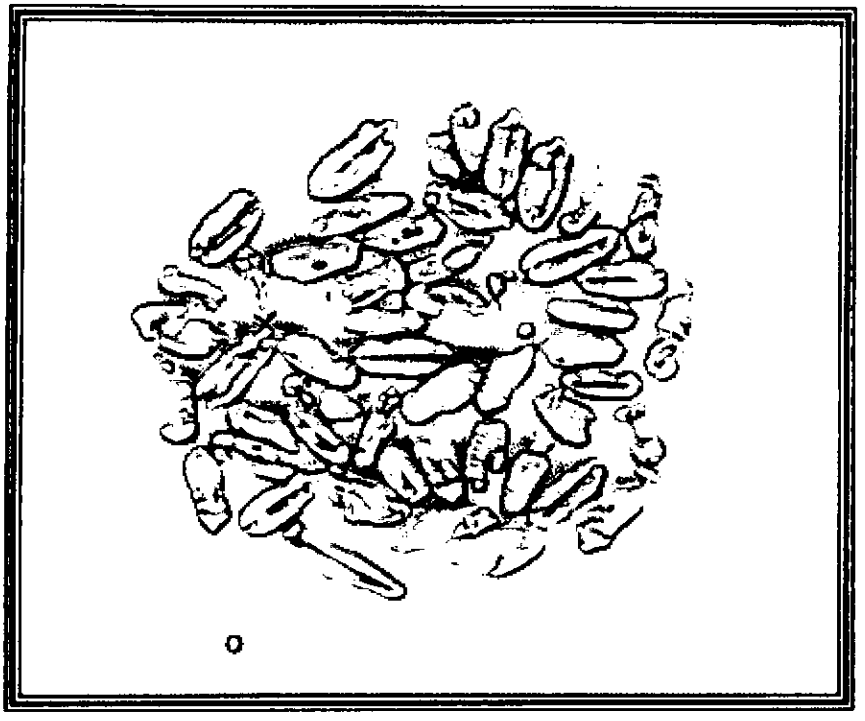


Plate 10. Discoloured seed sorted out from farmers' saved wheat seed.

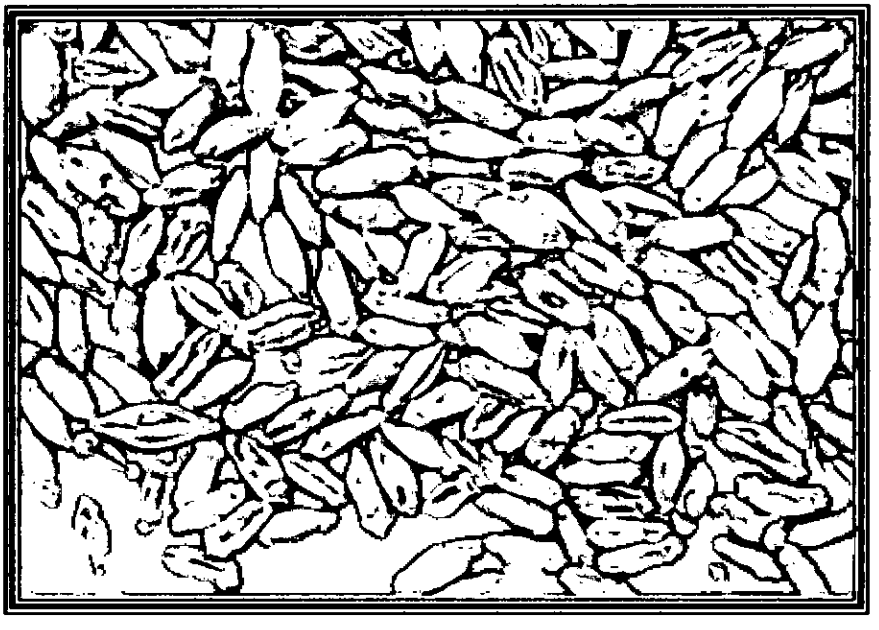


Plate 11. Shriveled seed sorted out from farmers' saved wheat seed.

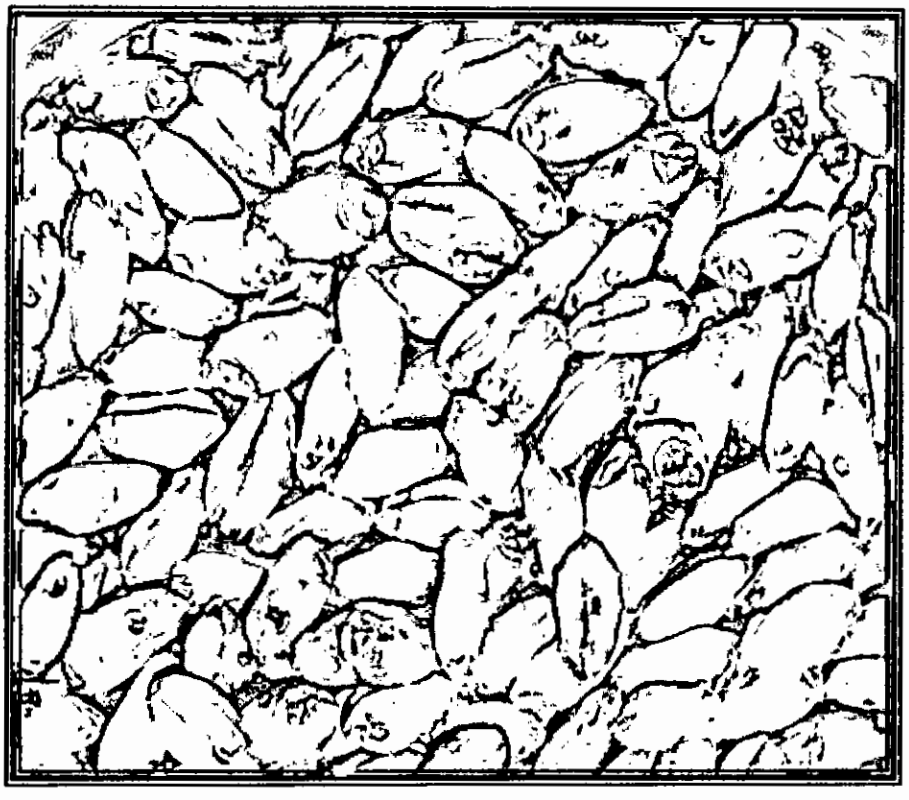


Plate 12. Insect damaged seed sorted out from farmers' saved wheat seed.

Highest percent of discolored seeds were noted at Dinajpur (3.82%), followed by Mymensingh (3.37%) and Sherpur (3.27%); while the lowest was encountered at Rajbari (0.37%).

The prevalence of shriveled seed and insect damaged seed ranged from 0.90 – 4.80% at Rajbari and Ishurdi, and 0.02%–7.35% at Rajbari and Atghoria, respectively.

Table 2. Abnormal seeds recorded in farmers' saved wheat seed collected from different locations of Bangladesh

Location	Undersized seed (%)	Black pointed seed (%)	Discoloured seed (%)	Shriveled seed (%)	Insect damaged seed (%)
Atghoria	17.64 ef	5.98 f	0.92 g	1.06 g	7.35 a
Dinajpur	19.22 de	10.71 c	3.82 a	3.55 b	1.59 cd
Faridpur	19.48 d	3.38 g	1.59 d	2.24 de	1.27 de
Ishurdi	17.49 f	8.07 d	2.58 c	4.80 a	1.02 e
Jhikargasa	16.52 g	25.12 b	1.36 f	1.68 f	0.51 f
Koshba	24.38 c	6.04 f	1.47 e	3.05 c	3.20 b
Mymensingh	31.61 ab	3.44 g	3.37 b	2.27 de	3.30 b
Rajbari	18.50 h	10.62 c	0.37 h	0.90 g	0.02 g
Sherpur	33.01 a	28.53 a	3.27 b	3.27 d	1.96 c
Ulipur	29.55 b	7.09 e	2.55 c	2.03 e	0.42 f
LSD (P≥0.01)	0.23	0.15	0.04	0.12	0.15
CV (%)	4.62	4.18	2.69	6.64	9.70

** = 1% level of significance

Analysis was performed after square root transformation of the % data.

4. 4. 'Clean' or 'Best' (Apparently healthy) Seed

Average percentage of 'Clean' or 'Best' (Apparently healthy) seed (Plate 13) recovered from farmers' saved seed ranged from 27.64 – 83.80% with respect to location of seed collection. Out of the ten locations, four had more than 60.00% 'Clean' or 'Best' seed. The four locations were – Dinajpur (83.80%), Ishurdi (69.96%), Mymensingh (68.62%) and Sherpur (63.03%), while the lowest quantity of 'Best' or 'Clean' seed was recovered from Ulipur (27.64%). The 'Clean' seed sorted out from the rest five locations ranged from 51.76 – 57.41%. On an average, 58.48% Best seed was recovered from farmer saved seed by manual seed cleaning (Fig. 3).

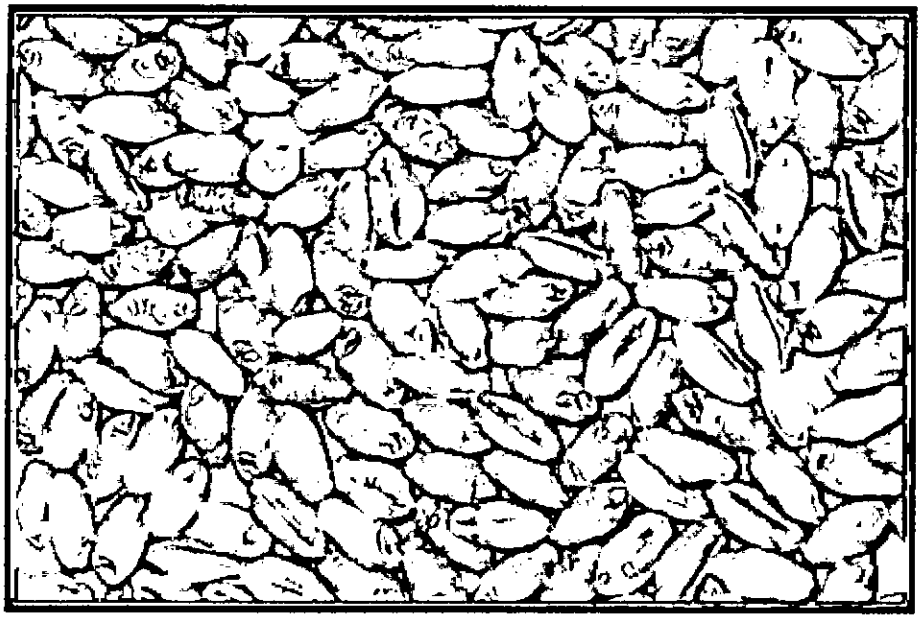


Plate 13. 'Clean' or 'Best' seed sorted out from farmers' saved wheat seed.

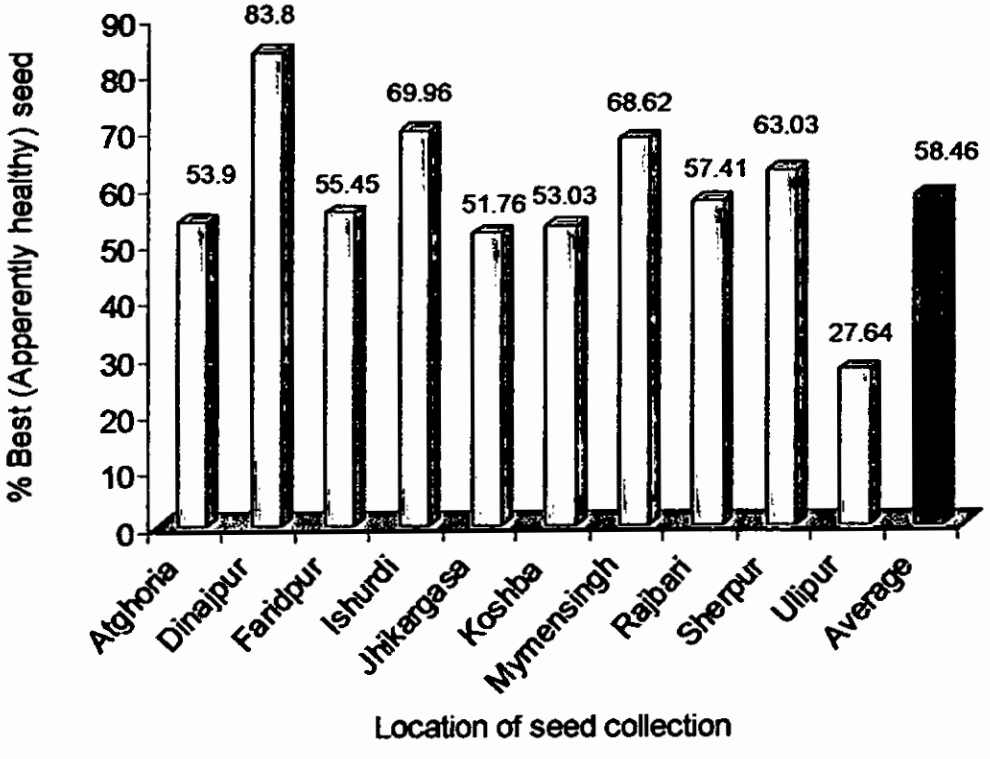


Fig. 3. 'Best' or apparently healthy wheat seed recovered from farmers saved wheat seed collected from ten different locations of Bangladesh.

4. 5. 1000 Seed Weight

Weight of 1000 seeds of the five selected wheat seed categories recorded in the present study are presented in Fig. 4. Of the five seed categories, 1000 'Best seed' had the highest weight (40.63g), followed by farmer saved seed (34.01g), black pointed seed (31.38g) and shriveled seed (21.54g). Of the five categories, undersized seed was the lightest; 1000 seeds of this category weighed 19.99g only.

Thousand seed weight of the six categories of black pointed seed decreased with the increase in black point severity (Fig. 5). The highest weight of 1000 seeds was recorded in Grades 0 i.e. wheat seed without black point; while the lowest weight was observed in Grade 5. It was also observed from the Figure that 1000 seed weight decreased progressively from Grade 1 to Grade 5.

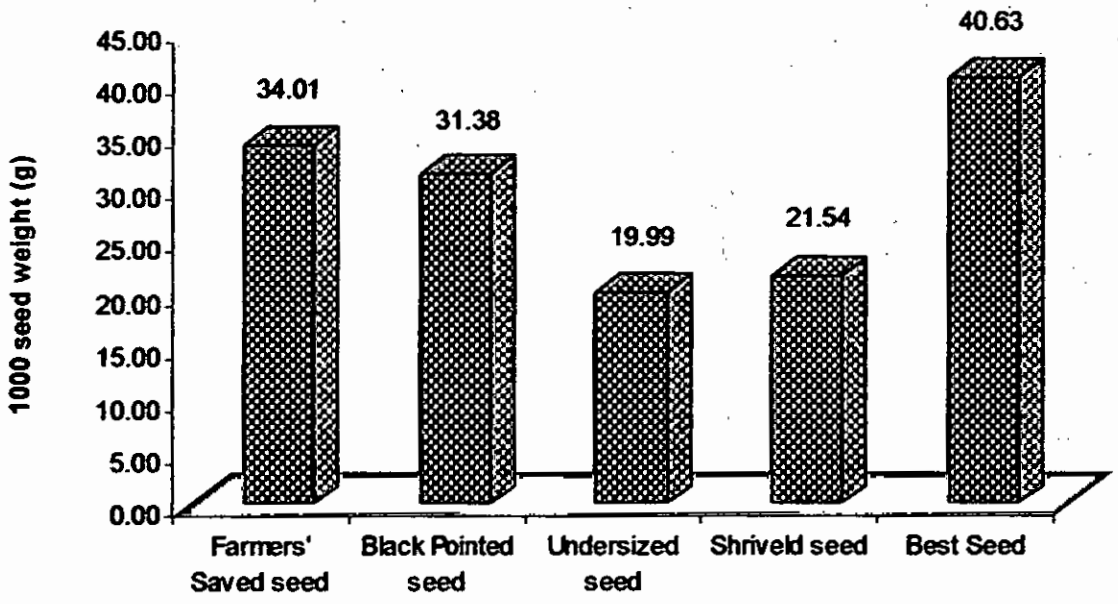


Fig. 4. Thousand seed weight of different categories of wheat seed

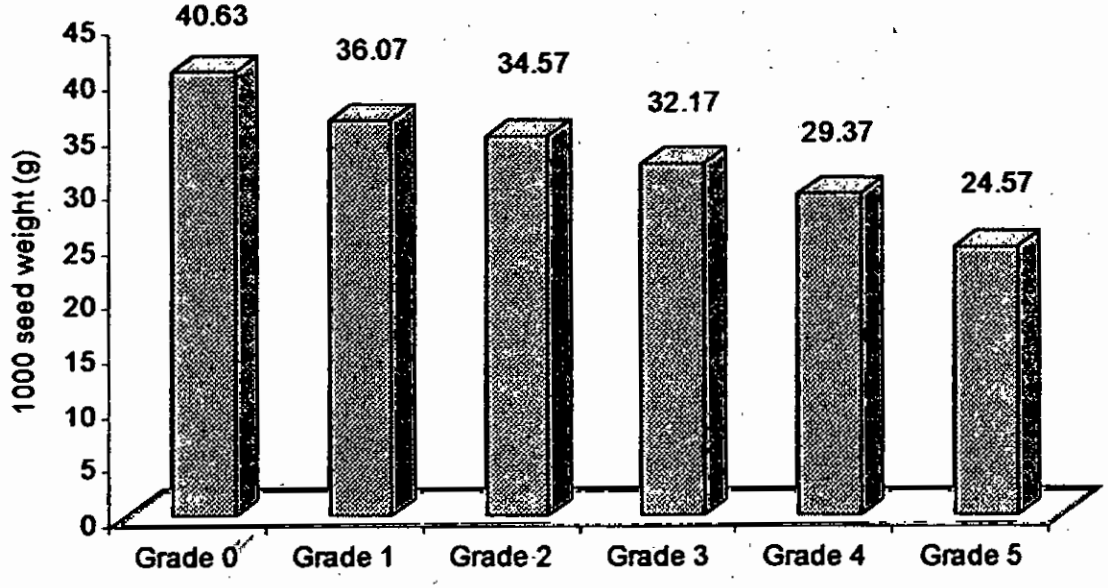


Fig. 5. Thousand seed weight of different categories of black pointed wheat seed

4.6. Prevalence of fungi

Thirteen species of fungi representing 11 genera recorded by blotter method from unclean (farmers' saved seed) and clean wheat seed obtained from 10 different locations of Bangladesh are presented in Appendix-2. The 13 fungi encountered were *Alternaria tenuis, Alternaria longissima, Aspergillus flavus, Aspergillus niger, Bipolaris sorokiniana, Chaetomium globosum, Cladosporium cladosporoides, Curvularia lunata, Fusarium oxysporum, Penicillium sp., Phoma sp., Rhizopus nigricans* and *Trichothecium roseum.* All the 13 fungal species were found in unclean seed, while in clean seed 10 fungal species (except *A. longissima, C. cladosporoides* and *Phoma* sp.,) were encountered.

4. 7. Prevalence of target pathogenic fungi

Out of the total 13 fungal species recorded in the present study five fungi- *B. sorokiniana* (Plate 14), *A. tenuis* (Plate 15), *C. lunata* (Plate 16), *F. oxysporum* (Plate 17) and *A. flavus* (Plate 18) were designated as the target pathogenic fungi as they are the established pathogens of wheat. The average percent seed borne infection of the five target pathogenic fungi encountered in unclean and clean wheat seeds are shown in Fig. 6. Unclean seed yielded higher percent of seed borne infection of all the five selected pathogens compared to clean seed. In

case of all the five test pathogens more than 30.00% reduced seed-borne infection was recorded in clean seed compared to unclean. Of the five selected pathogens, *B. sorokiniana* had the highest percent of seed borne infection both in unclean and clean seed, followed by *A. tenuis, C. lunata,* and *F. oxysporum,* while *A. flavus* had the lowest incidence. It can also be seen from the Fig. 6 that occurrence of *B. sorokiniana* was three to six times higher than *A. tenuis, C. lunata* or *F. oxysporum* and about 15 times higher than *A. flavus*.

A

B

Plate 14. A. Growth of *Bipolaris sorokiniana* on wheat seed incubated In blotter (X 45)

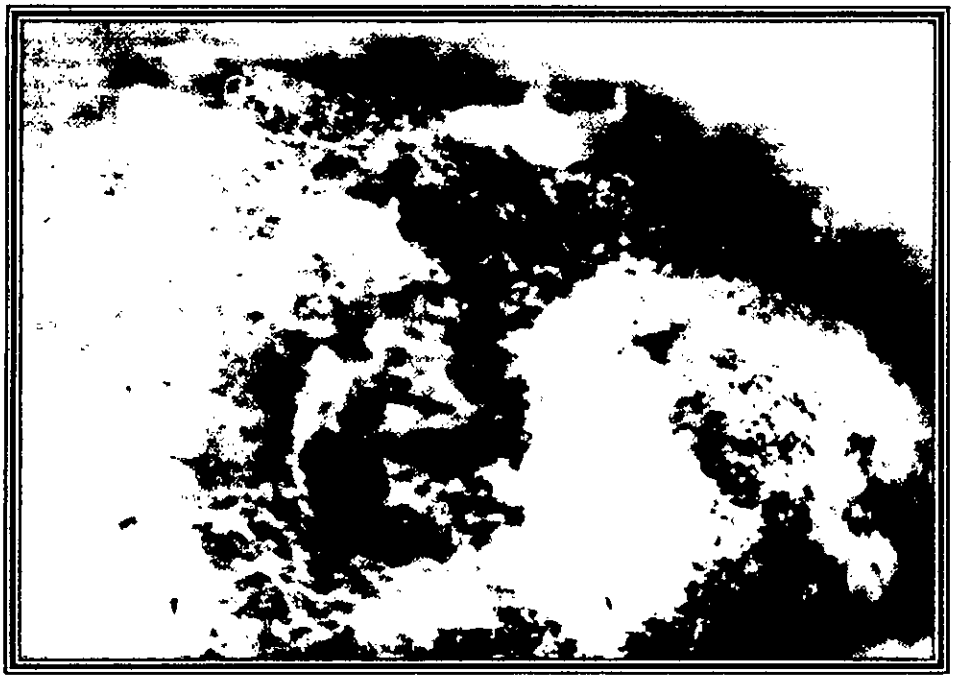
B. Conidia of *Bipolaris sorokiniana* (X 100)

A

B

Plate 15. A. Growth of *Alternaria tenuis* on incubated wheat seed in blotter (X 45)

B. Conidia of *Alternaria tenuis* (X 100)

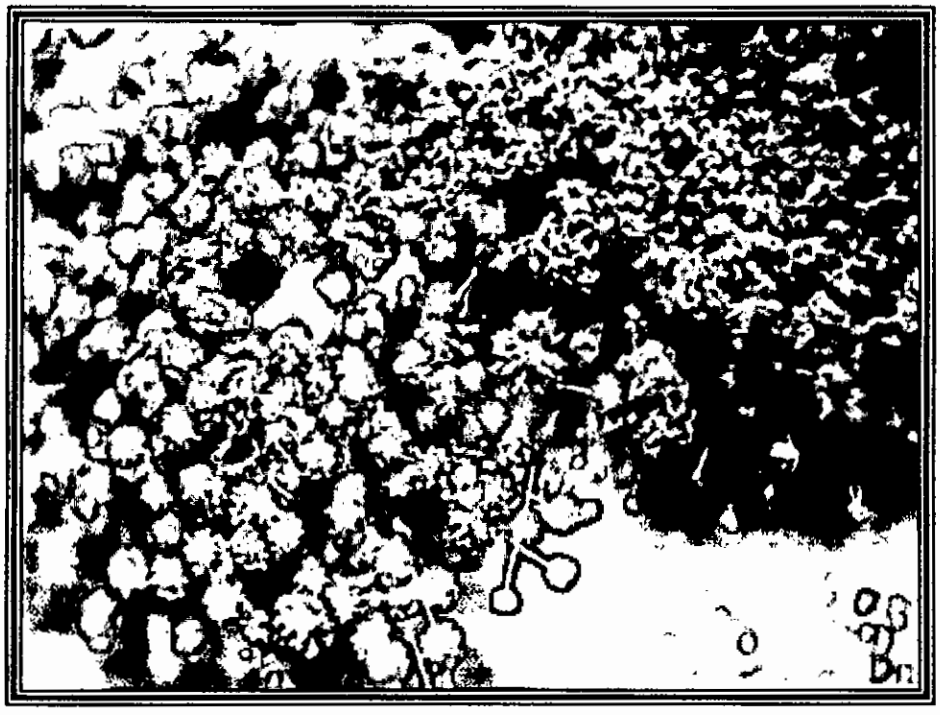


A

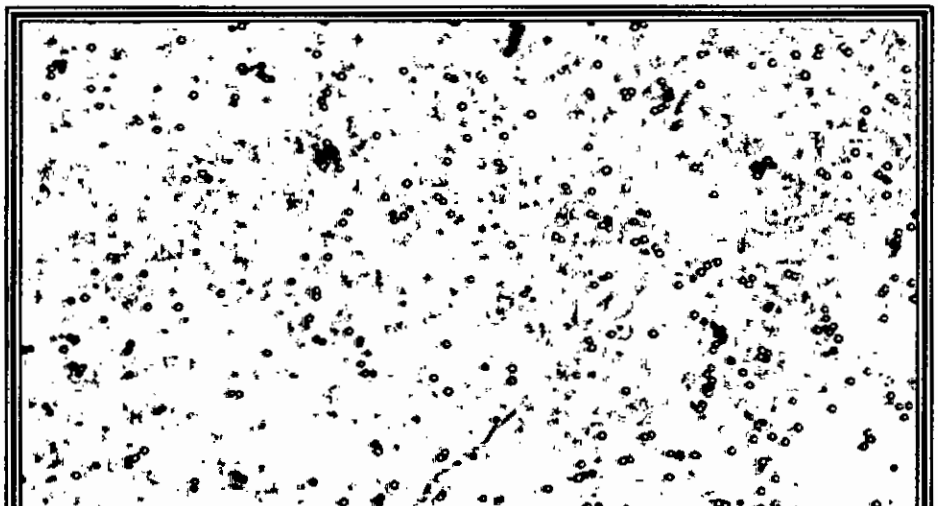
B

Plate 17. A. Growth of *Fusarium oxysporum* on incubated wheat seed in blotter (X 40)

B. Conidia of *Fusarium oxysporum* (X 100)



A



B

Plate 18. A. Growth of *Aspergillus flavus* on incubated wheat Seed in blotter (X 80)

B. Conidia of *Aspergillus flavus* (X 400)

The prevalence of the individual target fungal pathogens varied with respect to location of seed collection and seed cleanliness. Occurrence of all the five target fungi was always higher in unclean seed compared to clean seed at all the locations. Of the five target pathogens, *B sorokiniana* was the most predominant fungus, followed by *A. tenuis, C. lunata, F. oxysporum* and *A. flavus* in both unclean and clean seeds. The incidence of the fungus ranged between 37.75-84.88% and 30.00-66.00% in unclean and clean seed, respectively. The highest as well as the lowest occurrence of *B. sorokiniana* both in unclean and clean seed were recorded at Sherpur (84.88% and 66.00%) and at Faridpur (37.75 and 27.50%) (Table 3 and 4).

In unclean seed, the highest count of *A. tenuis* was recorded at Dinajpur (25.50%), followed by Sherpur (23.63%), Jhikargasa (22.88%), Atghoria (22.38%) and Koshba (19.50%), while the lowest was encountered at Faridpur (15.50%), whereas the highest and the lowest record of the fungus in clean seed was noted in Dinajpur (20.25%) and Rajbari (7.30%), respectively (Table 3 and 4).

The highest and the lowest prevalence of *C. lunata* in unclean seed was observed at Ulipur (36.25%) and Koshba (8.88%), respectively (Table 3). Similarly, the maximum incidence of the pathogen in clean seed was

recorded at Ulipur (22.88%) and the minimum (4.00%) at Koshba (Table 4).

In unclean seed, the highest seed-borne infection of *F. oxysporum* was found at Koshba (30.25%), while the lowest (4.75%) was detected at Ishurdi (Table 3). In case of clean seed, the highest and the lowest occurrence of the target pathogen was encountered at Dinajpur (13.88%) and Ishurdi (2.87%), respectively (Table 4).

The occurrence of *A. flavus* in unclean seed ranged from 1.62% at Rajbari to 26.38% at Ulipur (Table 3), while in clean seed the lowest percent (0.62%) of infection was observed at Rajbari and the highest (17.75%) at Ulipur (Table 4).

Table 3. Prevalence of five target pathogenic fungi in unclean farmers' saved wheat seed collected from 10 different locations of Bangladesh.

Location	% seed borne infection of				
	B. sorokiniana	*A. tenuis*	*C. lunata*	*F.oxysporum*	*A. flavus*
Atghoria	47.38 ef	22.38 abc	35.88 a	18.75 cd	16.2 b
Dinajpur	66.75 bc	25.50 a	20.75 bc	22.00 bc	2.87 e
Faridpur	37.75 g	15.50 d	11.50 cd	13.88 de	3.50 e
Ishurdi	67.63 b	19.38 abcd	13.50 bcd	4.75 f	1.87 e
Jhikargasa	80.75 a	22.88 abc	20.25 bc	15.25 cde	10.00 c
Koshba	54.38 de	19.50 abcd	8.875 d	30.25 a	3.12 e
Mymensingh	39.50 fg	16.50 cd	11.13 cd	10.75 ef	4.87 de
Rajbari	68.88 b	18.75 bcd	13.75 bcd	11.50 ef	1.62 e
Sherpur	84.88 a	23.63 ab	22.88 b	17.13 cde	8.62 cd
Ulipur	59.00 cd	19.38 abcd	36.25 a	29.13 ab	26.38 a
LSD ($p \geq 0.01$)	8.26	6.49	10.48	7.22	5.11
CV (%)	6.94	16.29	27.46	21.26	32.94

Table 4. Prevalence of five target pathogenic fungi in clean farmers' saved wheat seed collected from 10 different locations of Bangladesh

Location	% seed borne infection of				
	B. sorokiniana	*A. tenuis*	*C. lunata*	*F.oxysporum*	*A. flavus*
Atghoria	30.00 c	15.50 ab	15.38 bc	8.87 b	4.62 b
Dinajpur	37.63 bc	20.25 a	10.13 cd	13.88 a	1.50 bc
Faridpur	27.50 c	10.50 bc	4.12 e	7.87 b	2.00 bc
Ishurdi	39.13 bc	13.75 abc	7.25 de	2.87 c	1.12 bc
Jhikargasa	63.00 a	14.25 abc	16.13 b	9.50 ab	4.75 b
Koshba	32.50 bc	7.750 c	4.00 e	10.00 ab	1.00 bc
Mymensingh	33.75 bc	11.75 bc	6.00 de	6.00 bc	2.87 bc
Rajbari	49.38 ab	7.625 c	4.75 de	6.37 bc	0.62 c
Sherpur	66.00 a	16.88 ab	17.88 ab	8.87 b	4.75 b
Ulipur	29.63 c	12.25 bc	22.88 a	10.25 ab	17.75 a
LSD ($p \geq 0.01$)	17.97	7.09	5.45	4.69	3.98
CV (%)	22.45	27.73	25.87	28.36	49.59

4.8. Germination

Percentage of normal seedling (Plate 19 a), abnormal seedling (Plate 19 b) and dead seed (Plate 19 c) recorded in unclean and clean seeds in the germination test varied significantly depending on the locations and cleanliness of seed. In general, higher percentage of normal seedlings and lower percentage of abnormal seedlings and dead seeds were recorded in clean seed compared to unclean farmer saved seed at all the sites of seed collection (Figs. 7–9). The highest count of normal seedlings was obtained in unclean seed at Faridpur (84.38%) followed by Atghoria (81.13%), whereas the lowest incidence of normal seedlings was observed in clean seed at Ulipur (49.25%). On other hand, the highest count of normal seedlings in clean seed was recorded at Atghoria (93.50%), followed by Faridpur (91.88%), while, the lowest count (80.00%) of normal seedlings was observed at Jhikargasa (Fig. 7).

In unclean seed, the highest count of abnormal seedlings was found at Ulipur (13.70%), followed by Koshba (9.75%) and Dinajpur (8.37%), whereas the lowest incidence of abnormal seedlings was observed at Jhikargasa (5.37%). In case of clean seed, the highest count of abnormal seedlings was obtained at Koshba (9.75%), followed by Jhikargasa

(7.50%), while the lowest incidence (2.87%) of abnormal seedlings was recorded at Ulipur (Fig. 8).

In unclean seed' the highest count of dead seed was found at Rajbari (19.00%) followed by Ishurdi (18.25%), while the lowest incidence of dead seeds was encountered at Faridpur (8.75%). In case of clean seed, the maximum percentage of dead seed was obtained at Sherpur (12.75%), followed by Jhikargasa (12.50%) and Ishurdi (11.75%), while the minimum percentage of dead seed (2.37%) was recorded at Faridpur and Atghoria (Fig. 9).

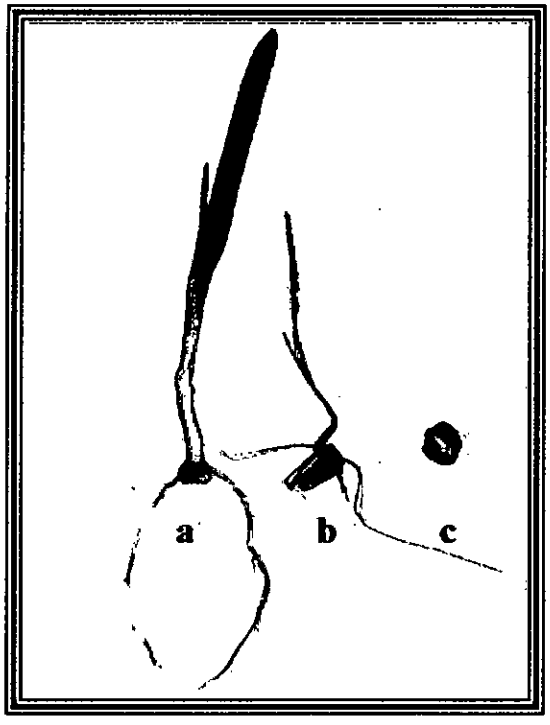


Plate 19. (a) Normal seedling
(b) Abnormal seedling and
(c) Dead seed

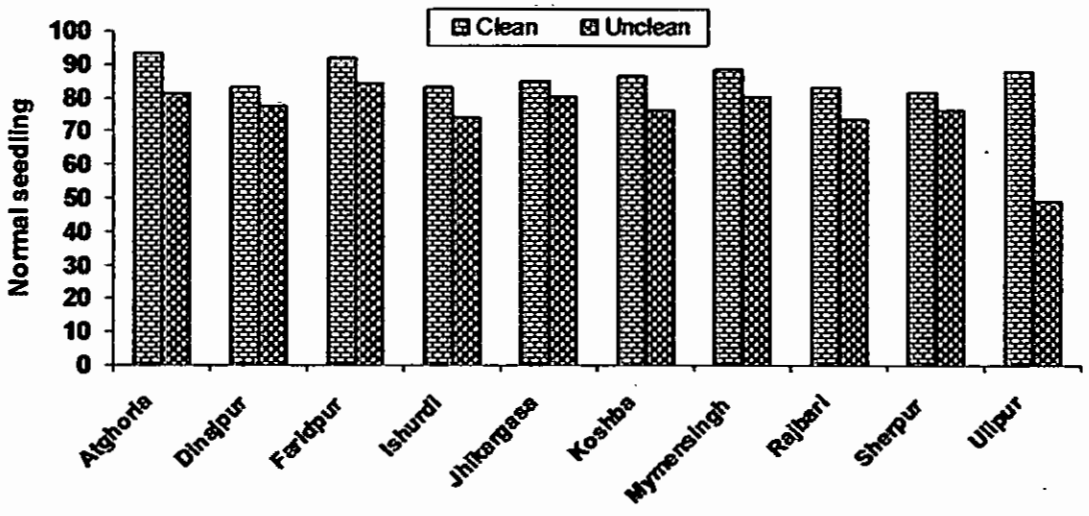


Fig.7. Percentage of normal seedling in clean and unclean wheat seeds collected from 10 different locations of Bangladesh

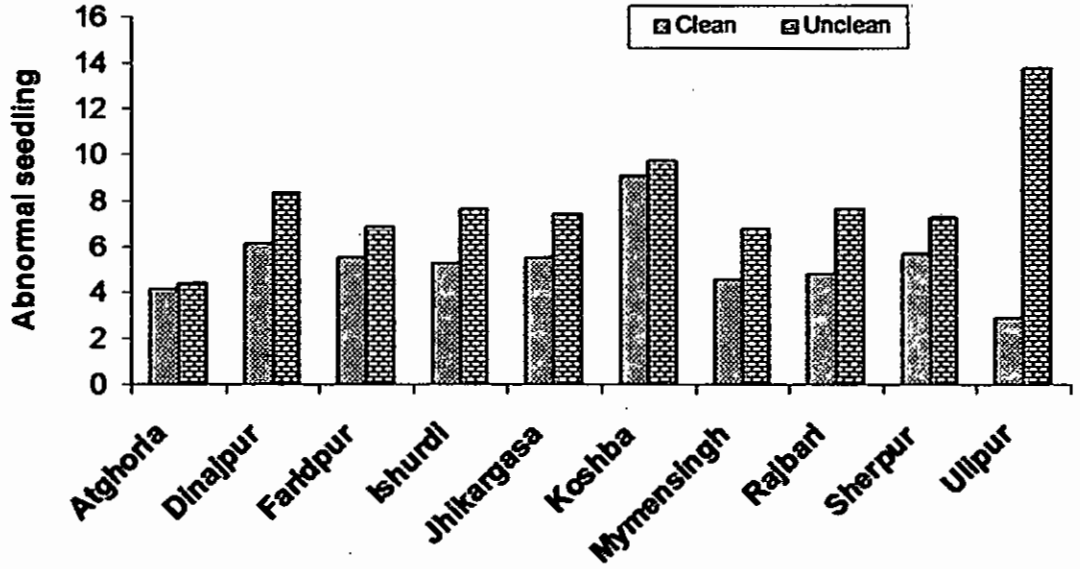


Fig.8. Percentage of abnormal seedling in clean and unclean wheat seeds collected from 10 different locations of Bangladesh

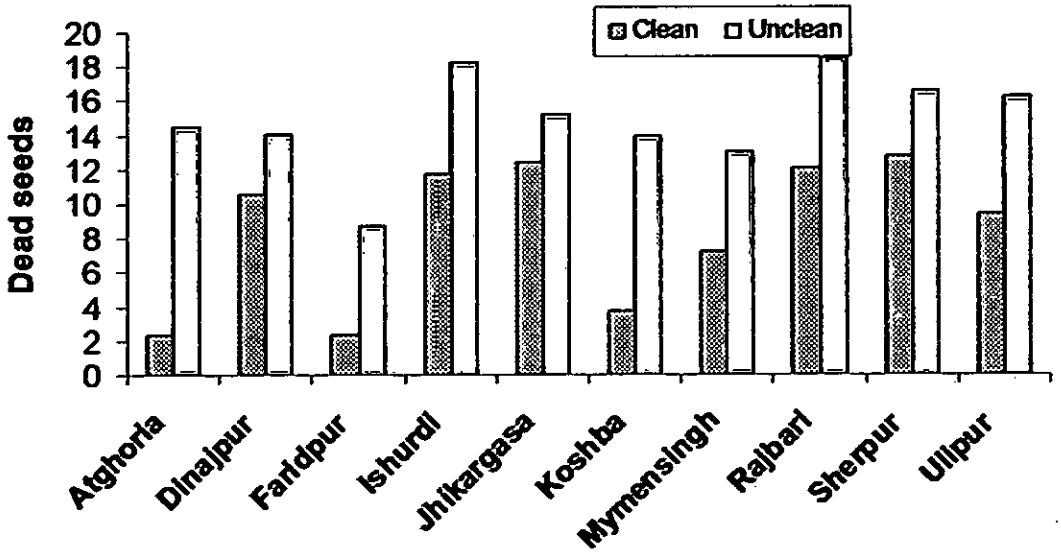


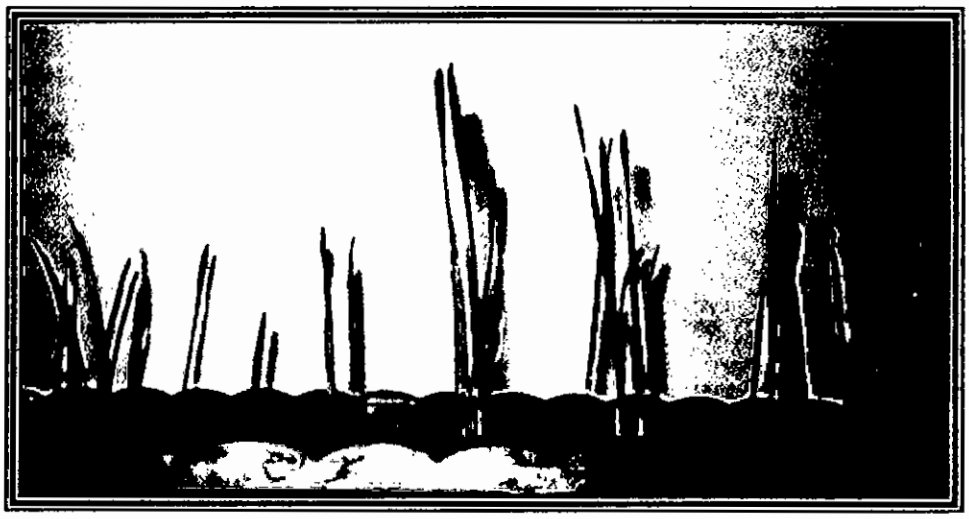
Fig.9. Percentage of dead seeds in clean and unclean wheat seeds collected from 10 different locations of Bangladesh

4.9.1. Seedling vigour observed in different categories of wheat seeds

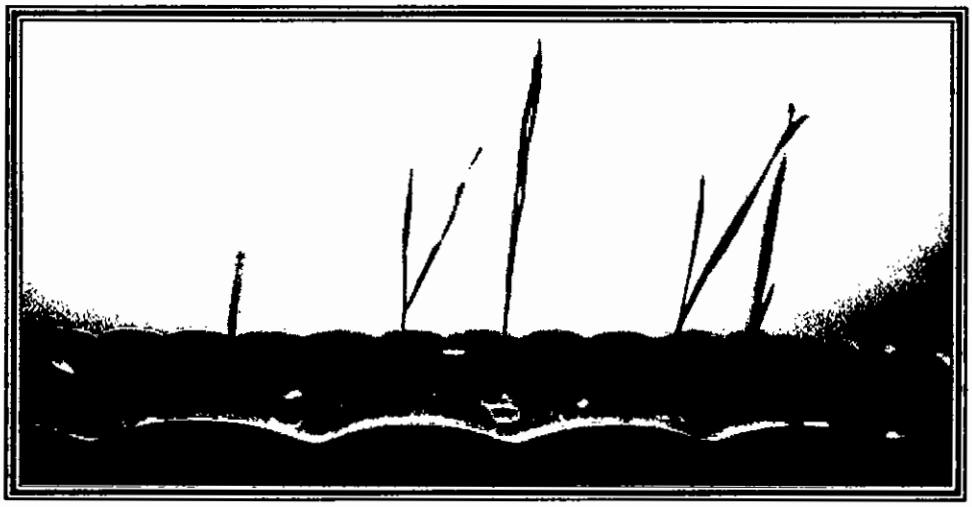
The root length, shoot length, germination and seedling vigour index recorded in five different categories of wheat seeds are presented in Table 5 and Plates 20-21. Root length, shoot length and germination varied significantly among the categories of seeds. 'Best' or 'Clean'seed always gave the highest root length, shoot length, germination and vigour index and the lowest root length, shoot length, germination and vigour index were recorded in undersized seed. It was also observed from the Table that farmer saved seed produced less root length, shoot length, germination and vigour index than the best seed. The root length and shoot length of black pointed seed and undersized seed did not differ significantly but the germination and vigour index showed wide variation. Shriveled seed produced higher root length, shoot length, germination and vigour index compared to undersized seed.

Table 5. Seedling vigour recorded in different categories of wheat seed

Seed categories	Root length (cm)	Shoot length (cm)	Germination (%)	Vigour index (VI)
Farmer saved seed	16.06 ab	11.77 a	79.17 a	2195.0
Black pointed seed	11.55 b	8.61 b	56.08 b	1132.0
Undersized seed	11.44 b	7.707 b	24.08 c	463.7
Shriveled seed	16.97 ab	7.723 b	36.17 bc	884.8
Best seed	20.39 a	12.40 a	95.25 a	3124.0
LSD (P≥0.01)	6.10	3.13	20.75	-
CV (%)	14.17	11.85	13.02	-

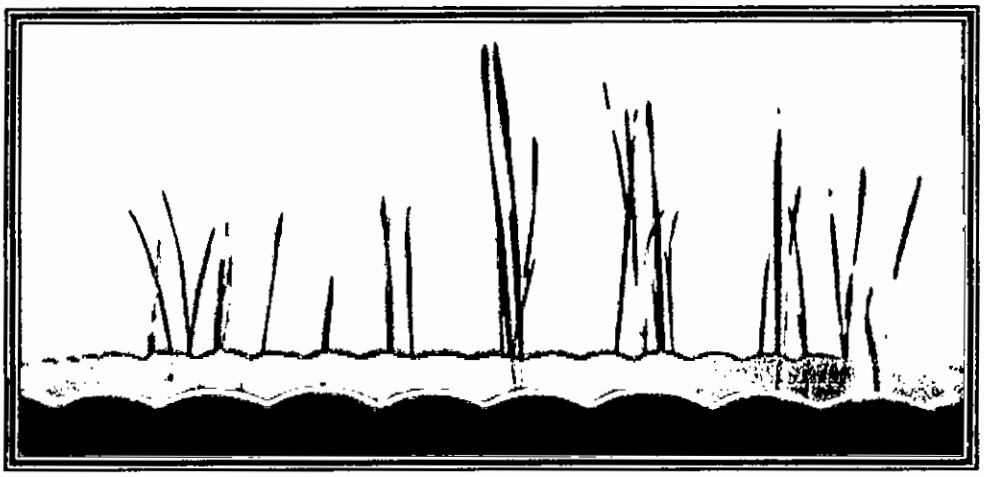


A

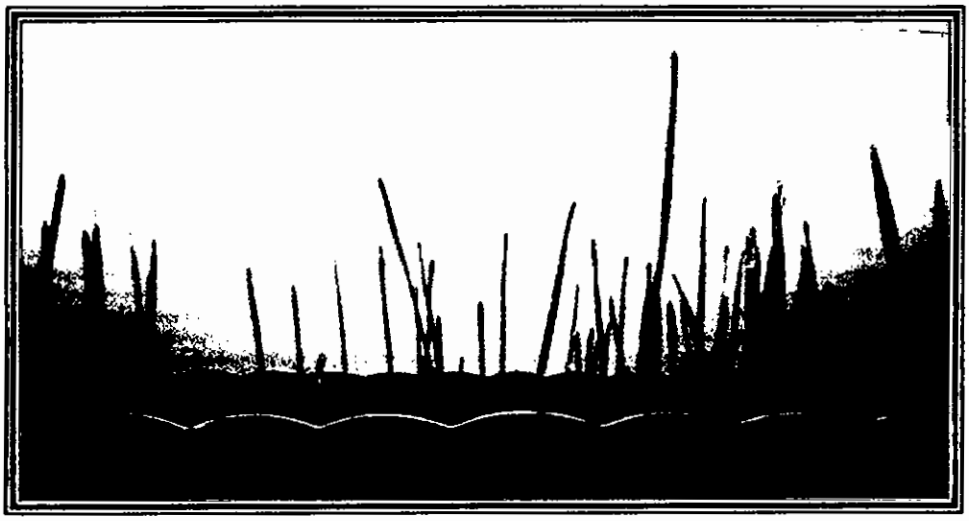


B

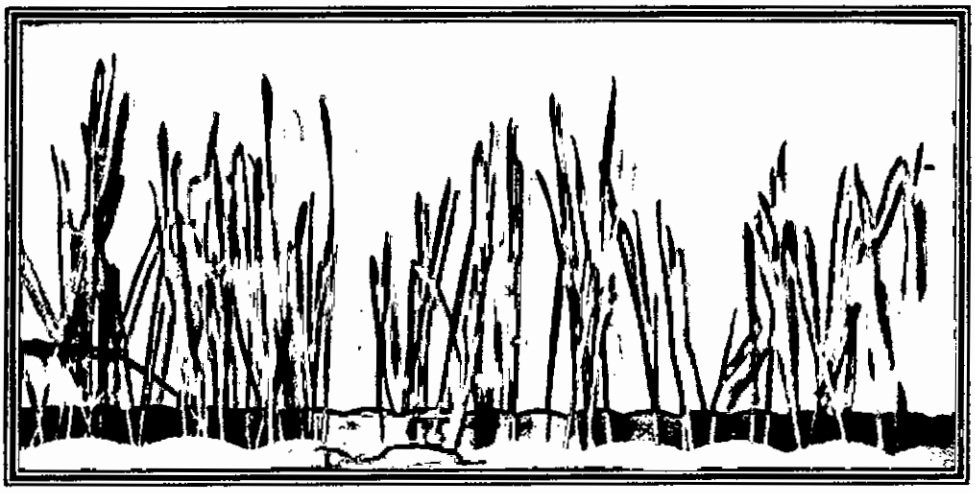
Plate 20. Germination of different categories of wheat seeds
A. Farmer saved seed B. Black pointed seed



A



B



C

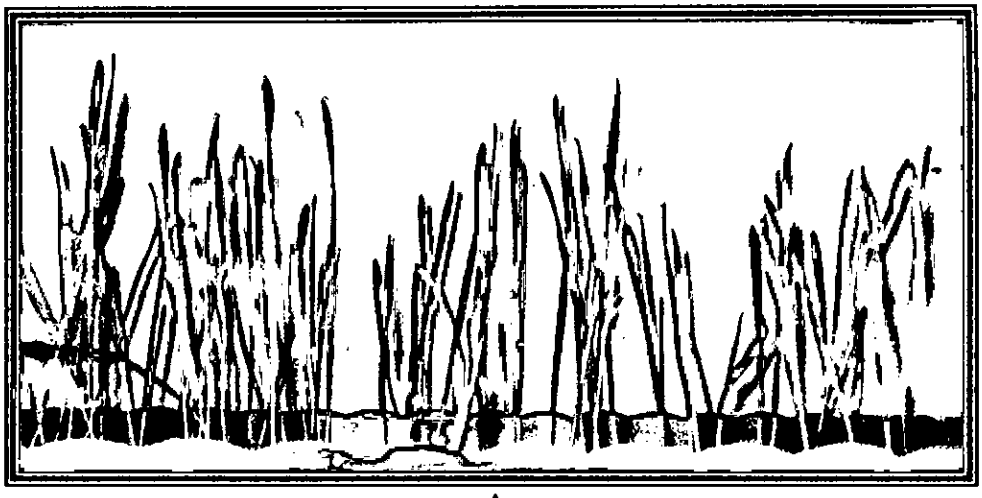
Plate 21. Germination of different categories of wheat seeds
A. udersized seed B. shriveled seed C. Best seed

4.9. 2. Seedling vigour observed in different grades of black pointed seeds

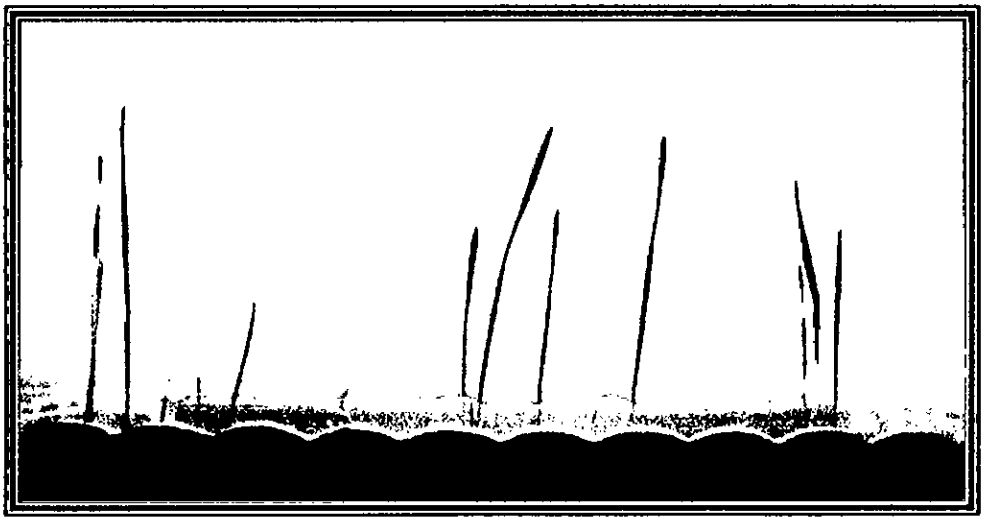
The results of the seedling vigour index of the black pointed wheat seeds graded in 0-5 scale are presented in Table 6 and Plates 22-23. Root length, shoot length and germination varied significantly with respect to different grades of seeds. Seeds belonging to the '0' (zero) grade (i.e. seed without black point) always gave the longest root length (17.70cm), and shoot length (15.40cm), and the highest germination (90.08%) and vigour index (2012.0), while the shortest root (7.20cm) and shoot length (4.40cm) and lowest germination (2.25%) and vigour index (27.94) were recorded in Grade 5 black pointed seed. Progressively lower root length, shoot length, germination and vigour index were recorded in Grade 1- 5 compared to '0' black point grade.

Table 6. Seedling vigour recorded in different grades of black pointed seeds

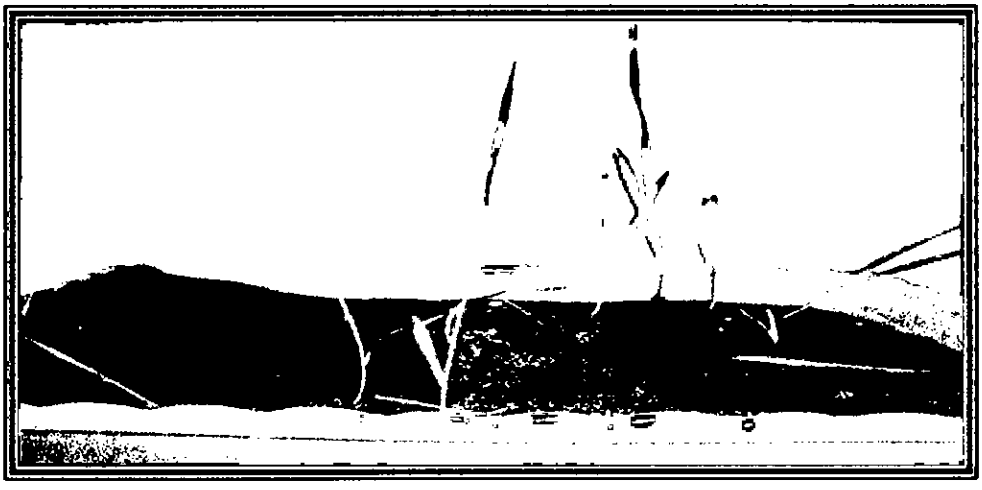
Black point grade	Root length (cm)	Shoot length (cm)	Germination (%)	Vigour index
0	17.70 a	15.40 a	90.08 a	2012.0
1	17.23 a	10.84 ab	50.58 b	1428.0
2	12.33 ab	9.06 ab	35.25 c	754.9
3	12.07 ab	6.03 b	15.25 d	238.6
4	9.47 ab	5.17 b	9.00 de	128.1
5	7.20 b	4.40 b	2.25 e	27.9
LSD (P≥0.01)	9.32	8.18	7.95	-
CV (%)	18.45	7.23	9.11	-



A

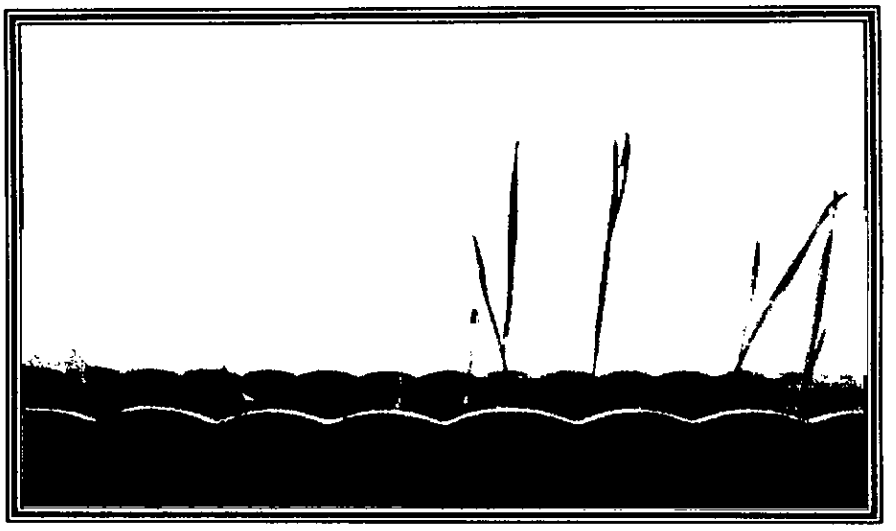


B

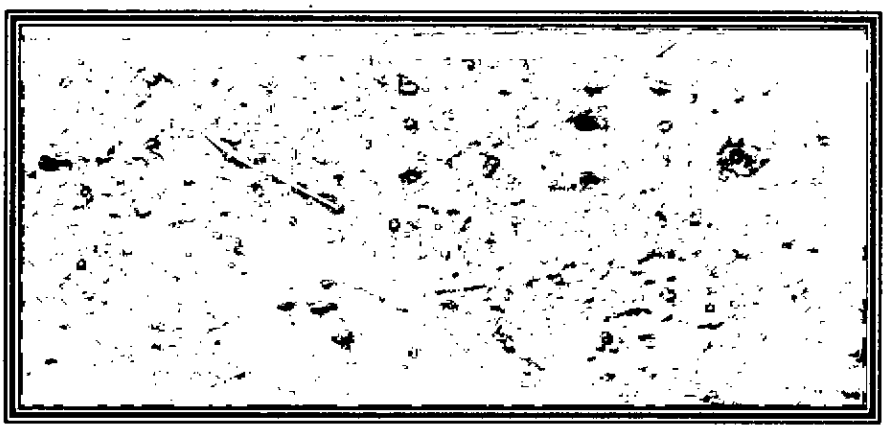


C

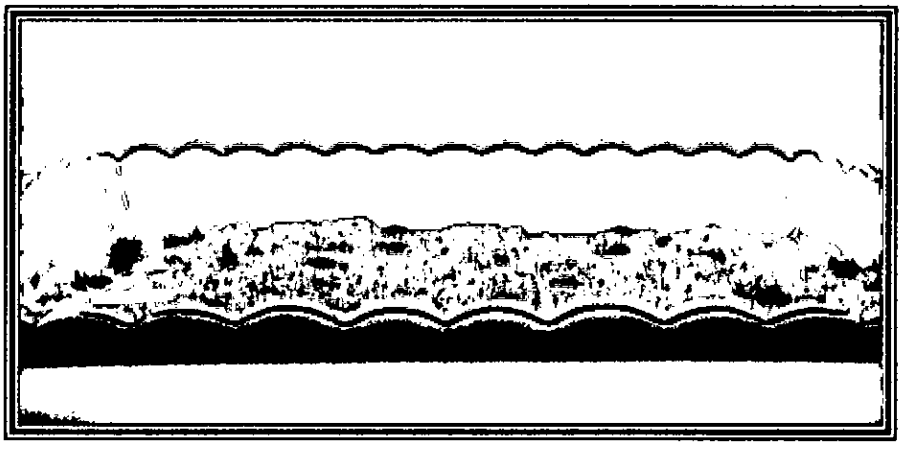
Plate 22. Germination of Black pointed seeds
A. Grade 0 B. Grade 1 C. Grade 2



A

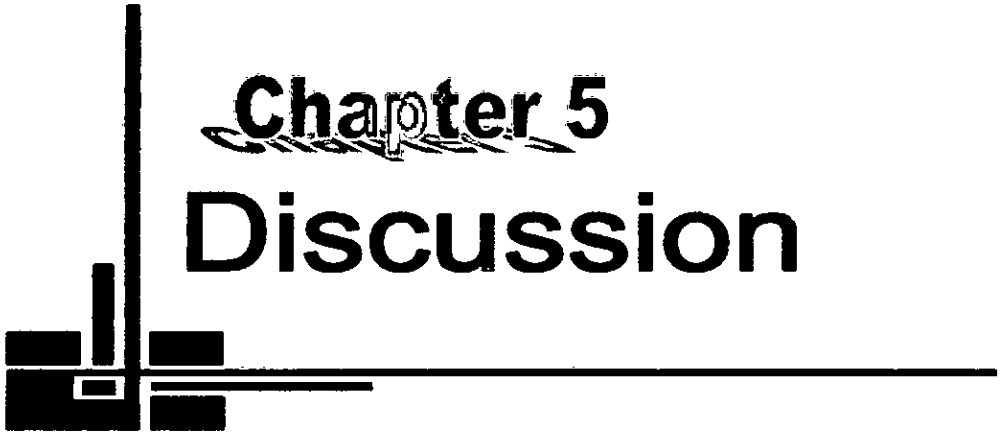


B



C

Plate 23. Germination of Black pointed seeds
A. Grade 3 B. Grade 4 C. Grade 5



Chapter 5
Discussion

5. DISCUSSION

The present study was undertaken to assess the status of seed health and quality of farmer saved wheat seed in Bangladesh with the ultimate objective for its improvement. Ten upazilas under nine old districts representing the major wheat growing areas of the country were included in the study. One hundred seed samples used for the study were collected from 100 different farmers representing 10 villages under each of the selected upazilas. Further, seeds were obtained from individual farmer's own seed store. This indicates the soundness of the research programme and planning.

From the results of the seed quality analysis, it is found that the average moisture content of farmer saved wheat seeds varied from 12.04 – 13.30%. Variation in moisture content of seeds of the individual farmers of the selected villages and upazilas at 10 locations under nine districts observed was probably due to the differences in the degree of drying of seeds. Again, this difference could be due to the ignorance of the farmers about the deleterious effect of high moisture content in seeds. Or, it could be due to the exposure of seeds to wet weather during pre-monsoon days when wheat crop is harvested and processed. The national standard for

moisture content of wheat seed is 12.00%. But, moisture content data obtained in the present study showed that all the hundred seed samples had higher moisture content than the national standard. High moisture content in seed prior to storage is not safe for health of stored seeds. Because, wheat seeds stored with higher moisture content is vulnerable to the attack of storage fungi and stored grain insects, which often cause considerable losses through reduction in germination (Christensen and Kaufmann, 1965; Islam *et al.* 1991).

Seed contaminants *viz.* weed seeds, insects, varietal mixture, seed with husk, other crop seeds and inert matter occurred in most of the samples collected from all the ten sites. These contaminants occurred in trace to appreciable extent depending on the type of contaminant and site of seed collection. This situation indicates that farmer saved wheat seed is not of good quality. Because, seeds of weed species may carry inocula of fungal pathogen like *B. sorokiniana*. Although no study has been made on the role of weeds grown in the wheat field in harbouring pathogens as alternate host, seeds of certain weed species found in farmer saved rice seed, which have been reported to serve as alternate hosts of *Bipolaris oryzae* and *Pyricularia oryzae* (Fakir and Mia, 2004).

Three species of storage insects - red flour beetle, rice moth and weevil have been encountered in farmer saved wheat seed in the present study.

Occurrence of as high as 7.35% insect damaged seed at certain location as seed contaminant (Table 2) further bears the testimony of deleterious effect of stored insects on the quality of farmer saved wheat seed.

Inert matter and seed with husk present in all the samples analysed may carry viable spores of seed- borne pathogenic fungi attacking wheat. This has not been investigated in wheat earlier or in the present study. But there is evidence that inert matter present in rice seed produced by the farmers' of Bangladesh can carry viable propagules of *B. oryzae*, *A. padwickii*; *Fusarium spp*, *Aspergillus spp.* and *Penicillium sp.* (Khokon *et al.*, 2005).Thus, the presence of inert matter in farmer saved seed poses risk as regard to contamination of seed lot by the propagules of pathogenic fungi. Therefore, studies need to be undertaken on the role of inert matter present in farmers' saved wheat seed in carrying pathogenic fungi.

Occurrence of good percentage of seed of other wheat varieties (varietal mixture) in farmer saved wheat seed in the present study indicates that farmers are not much careful about the varietal mixture in their saved seeds. Presence of seeds of other wheat varieties as well as seeds of other crops in a given seed lot may affect the health of the seed lot through mixture of seeds of susceptible varieties/crops with the saved seeds of a resistant variety.

Presence of five types of abnormal seeds *viz.* undersized seed, black pointed seed, discoloured seed, shriveled seed and insect damaged seed in the farmer saved seed further proves that the quality of our farmers' wheat seed is poor. Occurrence of abnormal seeds like black pointed, discoloured, undersized and shriveled seeds, poses threat of carrying leaf blight pathogen, *B. sorokiniana* and black point pathogens like *B. sorokiniana, A. tenuis, C. lunata* and *Fusarium* spp., which might be responsible for causing leaf blight disease in the field and discoloration and black point symptom to wheat seed. It has been demonstrated that creamy to pink coloured shriveled wheat seeds were infected by *Fusarium* spp. (Agarwal *et al.*, 1993). Kalaknnavar *et al.* (1989) also demonstrated that seedling vigour, specially root length and seedling growth was found to decrease with the decrease in seed size of wheat.

In an average, 58.48% clean or best (apparently healthy) seed could be recovered from the original farmer saved seed (Fig. 3). Thus, the study shows that more than 40% of the wheat seed produced by the farmers is discarded due to presence of abnormal seeds. This is an alarming situation in wheat seed production for Bangladesh. The situation demands more careful attention to seed crop management and processing of seeds after harvest specially during cleaning operations.

Thirteen fungi were detected in farmer saved or uncleaned seed, while 10 fungi, except *A. longissima, C. cladosporoides* and *Phoma sp.*, were encountered in the manually sorted out best or clean seed (Appendix 2). The cause of absence of the three fungi in clean seed was probably due to the fact that seed cleaning processes might have eliminated them from the original farmer saved seed.

Occurrence of the five target pathogenic fungi varied independently of each other with respect to the location of seed collection. Variation in the prevalence of seed-borne fungal pathogens with regard to geographic locations has been demonstrated earlier in a number of crops like rice, kaon, mustard, black gram, wheat, jute, chilli, zinnia, maize, barley, sorghum and cheena by different research workers in Bangladesh (Hossain *et al.*, 1977; Barma and Fakir, 1981; Dey and Fakir, 1988; Kabir and Fakir, 1988; Rahman *et al.*, 1988; Fakir and Islam, 1990; Basak *et al.*, 1991; Fakir and Halder, 1993; Sultana, 2004; Fakir and Mia 2004; Karim, 2005; Islam, 2005;). More than 30% population of each of the five target fungi was found to be reduced in clean seed in comparison with unclean seed at all the locations. This indicates that manual seed cleaning has significant impact on the reduction of the pathogenic fungi in farmer saved wheat seed. Such reduction in population of pathogenic fungi through manual seed cleaning has been observed in rice (Hossain

and Doullah, 1998; Rahman, 2000; Kabir *et al,*. 2005; Khokon *et al,*. 2005), egg plant (Meah, 2002; Hawlader, 2003 and Islam, 2005), mustard (Chowdhury, 1999) and jute (Fakir *et al.,* 1990). Reduced population of pathogenic fungi has also been encountered in apparently healthy wheat seed obtained through sieving (Siddique, 2003).

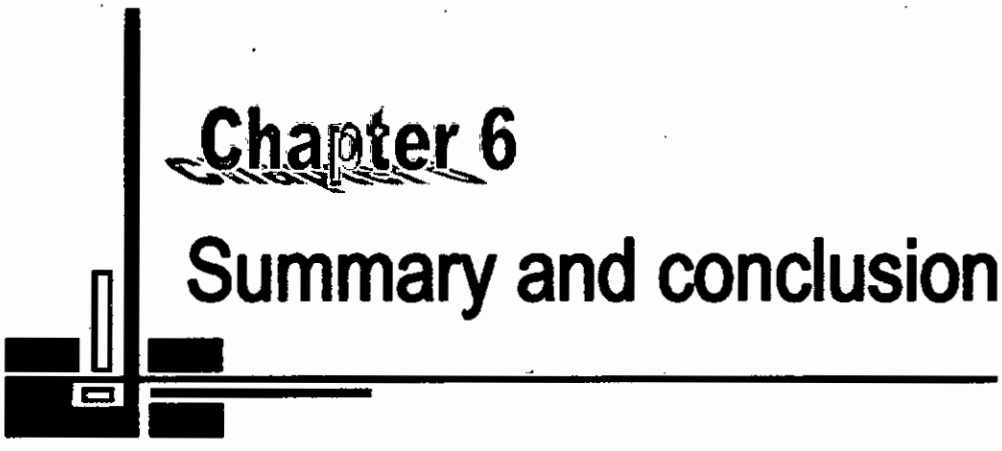
Of the five target fungal pathogens, *B. sorokiniana* was the most pre dominant. The pathogen had very high incidence ranging from 37.75-84.88% and 30.00-66.00% in unclean (Table 3) and clean seed (Table 4), respectively. *B. sorokiniana* is a dangerous seed borne pathogen responsible for causing devastating leaf blight and black point disease of wheat. Thus, quite high occurrence of the pathogen even in clean seed (30.00-66.00%) demands routine health test of wheat seeds prior to sowing.

Presence of seed-borne infection of the five target pathogenic fungi *B. sorokiniana*, *A. tenuis, C. lunata F. oxysporum* and *A. flavus* in the test seed samples depict that in no way the health status of wheat seed samples tested is satisfactory. Original saved wheat seeds carrying even the amount of inocula of the four pathogenic field fungi - *B. sorokiniana*, *A. tenuis, C. lunata* and *F. oxysporum* detected in the present analysis, if transmitted to the field, may create alarming disease outbreaks in the fields resulting heavy yield losses to the crop as well as hampering

quality healthy seed production. Occurrence of the dangerous storage fungus, *A. flavus* in the test seed samples also questions the keeping quality of farmer saved wheat seed in storage as the pathogen is known to cause germination loss in many crops (Christensen and Kaufman, 1965; Fakir *et al.*,1971; Miah and Fakir, 1989; Islam *et al.*,1991; Rahman and Mia. 1998; Fakir and Mia, 2004). The above mentioned facts can be supported from the germination test record in the present study, where original farmer saved seed had always poor germination, produced much lesser number of normal seedlings and resulted higher number of dead seed, abnormal or diseased seedlings and low seedling vigour (Figs. 7-9 and Tables 5-6). On the contrary, best seeds obtained from farmer saved original seed through physical cleaning by separation or removal of seed contaminants and abnormal seeds, had low seed borne infections of pathogenic fungi (Fig. 6) Best seeds in the germination test in natural soil resulted markedly higher percentage of normal seedlings, lesser percentage dead seeds, abnormal seedlings including diseased seedlings and high seedling vigour (Figs. 7-9 and Tables 5-6). Similar benefits of manual seed cleaning have been obtained in jute (Fakir *et al.*, 1990), mustard (Chowdhury, 1999), rice (Fakir and Mia, 2004) and egg plant (Islam, 2005).

The present study revealed that among the five different seed categories, 1000 clean or best seeds had the maximum weight, while the small undersized and shriveled seeds were lighter than seeds of other categories. Again, of the five categories of black point, Grade 5 (i.e. seeds most severely affected by black point) seeds were lighter in weight. Bolley (1913) had similar results while grading black pointed wheat grains. He reported that black pointed wheat grains were usually lighter in weight than normal best grains. In the present study, it was found that size and weight of wheat seeds had profound effect on germination and seedling vigour. Undersized smaller seeds and Grade 5 black point affected seeds showed lower germination and seedling vigour compared to bigger and heavier clean or best seeds (Tables 5-6). These results are consistent with the findings of other workers (Choudhury *et al.*, 1984; Khanum *et al.*, 1987; Kalakannavar *et al.*, 1989; Rahman and Islam, 1998; Khare and Satpute, 1999; Siddique, 2003).

From the foregoing discussions, it is clearly evident that the health status of farmer saved seed is poor. It is, therefore, suggested to provide proper training to the farmers for better seed crop management and proper storage of wheat seed. This will help to improve the seed health quality of wheat seed at farmers level and contribute for higher yield per unit area.



Chapter 6

Summary and conclusion

6. SUMMARY AND CONCLUSION

Health and quality status of farmer saved wheat seed grown in 2004-2005 was determined by seed health and quality analysis. One hundred seed samples of the wheat variety Kanchan were collected from 100 different farmers representing 10 villages under 10 upazilas located in nine major wheat growing districts. The ten locations were – 1) Atghoria, Pabna; 2) Dinajpur, Sadar; 3); Faridpur, Sadar 4); Ishurdi, Pabna 5) Jhikargasa, Jessore; 6) Koshba, Brahmanbaria; 7) Mymensingh, Sadar; 8) Rajbari, Sadar; 9) Sherpur, Bogra and 10) Ulipur, Kurigram.

The seed samples were analysed in the Departments of Plant Pathology, Sher-e-Bangladesh Agricultural University (SAU), Dhaka and Seed Pathology Centre (SPC), Bangladesh Agricultural University (BAU), Mymensingh. Seed samples were tested for moisture content by electronic digital moisture meter. Best or clean (Apparently healthy) seed was sorted out from the original farmer saved seed through elimination of seed contaminants and abnormal seeds by manual seed cleaning. Germination test was conducted in plastic trays with clean sand to record the normal seedlings, abnormal seedlings and dead seeds following the International Rules for Seed Testing. Seedling vigour was determined

following the formula of Baki and Anderson. Health analysis was carried out to detect seed borne fungal infections by Blotter method.

Seed quality analysis revealed that the average moisture content of the farmer saved wheat seed varied from 12.04 – 13.30% with respect to location of the seed collection. Six types of seed contaminants recorded in farmer saved seeds were - weed seeds, insects, varietal mixture, seed with husk, other crop seeds and inert matter. The seed contaminants varied significantly depending on the location of seed collection. Six species of weed seeds observed were - *Brassica kaber, Chenopodium album, Cyperus* sp., *Echinochloa crusgalli, Solanum nigrum, Polygonum hydropiper* and *Vicia sativa,* while the three species of insects encountered were *Tribolium casteneum*, *Corcyra cephalonica* and *Sitophillus oryza*.

Five types of abnormal seeds viz. black pointed seed, discoloured seed, undersized seed, shriveled seed and insect damaged seed recorded in this study varied in prevalence significantly with respect to location of seed collection. Among the different types of abnormal seeds, undersized seed was the most predominant followed by black pointed seed.

Average percentage of 'clean' or 'Best' seed recovered from farmers' saved seed ranged from 27.64 – 83.80% depending on the location of seed collection. Out of the ten locations, four had more than 60.00% 'Best' or 'clean' seed. On an average, 58.48% 'Best' seed was recovered from farmer saved seed by manual seed cleaning.

Out of five the seed categories, weight of 1000 'Best seed' had the highest weight followed by farmer saved seed, black pointed seed and shriveled seed, while undersized seed had the lowest weight.

Thousand seed weight of the six categories of black pointed seed (0-5 scale) decreased with the increase in black point severity. The highest weight of 1000 seeds was recorded in Grade 0, while the lowest weight was observed in Grade 5.

Thirteen fungi namely *Alternaria longissima, A. tenuis, Aspergillus flavus, A. niger, Bipolaris sorokiniana, Chaetomium globosum, Cladosporium cladosporoides, Curvularia lunata, Fusarium oxysporum, Penicellium sp, Phoma sp, Rhizopus nigricans, Trichothecium roseum* were detected in farmer saved wheat seed collected from 10 different locations of Bangladesh. Of the 13 fungi, five target pathogenic fungi viz. *B. sorokiniana, A. tenuis, C. lunata, F. oxysporum* and *A. flavus* detected

in the present study, varied in prevalence with respect to location of seed collection. Occurrence of all the five target pathogenic fungi was found always higher in unclean seed compared to clean seed at all the locations. Among the target fungi, *B. sorokiniana* was the most predominant fungus, followed by *A. tenuis, C. lunata, F. oxysporum* and *A. flavus* in both unclean and clean seeds. Normal seedlings, abnormal seedlings and dead seeds recorded in the germination test varied significantly depending on the location and cleanliness of seed. Higher percentage of normal seedlings and lower percentages of abnormal seedlings and dead seeds were always observed in clean seed compared to unclean seed at all locations. 'Clean' or 'best' seed resulted the highest germination (95.25%) and vigour index (3124.0), while the lowest germination (24.08%) and vigour index (463.7) were recorded in undersized seed. Among the different black point categories, Grade 0 i.e. seed without black point gave the highest germination (90.08%) and vigour index (2012.0), while the lowest germination (2.25%) and vigour index (27.9) were encountered in Grade 5.

Based on the present study the following conclusions may be drawn:

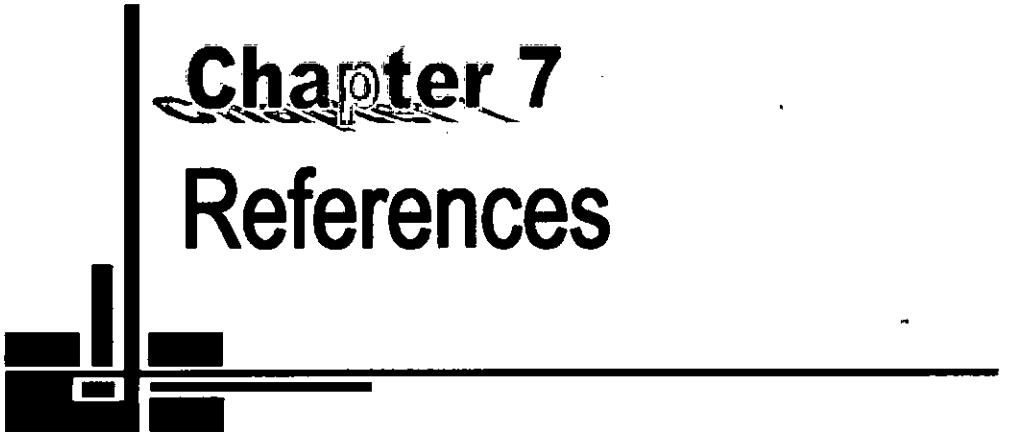
- Quality and health status of farmer saved wheat seed in Bangladesh is poor. Farmers' seed contains more moisture content, appreciable

quantity of seed contaminants, considerable amount of abnormal seeds and seed-borne infection of dangerous pathogenic fungi.

- About 60% 'Best' or clean (apparently healthy) wheat seed can be recovered from farmer saved seed through manual seed cleaning.

- Clean or best seed gives higher counts of normal seedlings and lesser percentage of abnormal seedlings and dead seeds over abnormal seeds like black pointed, undersized, discoloured and shriveled seeds. Clean seed also results higher seedling vigour index.

- Health and quality of farmer saved wheat seed can be improved markedly by manual seed cleaning.



Chapter 7
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APPENDIX

Appendix 1. List of farmers along with their address from where wheat seeds (cv. Kanchan) were collected

Sample No.	Farmer's Name	Address
1	Abdur Rashid	Bishelpur, Sherpur, Bogra
2	Md. Arshad Hossain	Bishelpur, Sherpur, Bogra
3	Md. Nobo Ali	Bishelpur, Sherpur, Bogra
4	Md. Shek Faruk	Bishelpur, Sherpur, Bogra
5	Md. Sadek Ali	Bishelpur, Sherpur, Bogra
6	Mohammad Sobuz	Bishelpur, Sherpur, Bogra
7	Md. Mokbul Hossain	Bishelpur, Sherpur, Bogra
8	Md. Alim Howladar	Bishelpur, Sherpur, Bogra
9	Md. Aftab Mia	Bishelpur, Sherpur, Bogra
10	Md. Rezaul Ali	Bishelpur, Sherpur, Bogra
11	Humayan Kabir	Chargasa, Koshba, Bramanbaria
12	Md. Ikbal Hossain	Chargasa, Koshba, Bramanbaria
13	Md. Abul Khayer	Chargasa, Koshba, Bramanbaria
14	Md. Rustam Miah	Chargasa, Koshba, Bramanbaria
15	Md. Sohel Mia	Chargasa, Koshba, Bramanbaria
16	Md. Sobed Ahmmed	Chargasa, Koshba, Bramanbaria
17	Md. ishak Mia	Chargasa, Koshba, Bramanbaria
18	Al Amin Kha	Chargasa, Koshba, Bramanbaria
19	Md. Alam Mia	Chargasa, Koshba, Bramanbaria
20	Shah Alam	Chargasa, Koshba, Bramanbaria
21	Surmot Ali	Chehelgazi, Dinajpur Sadar,
22	Giash uddin	Chehelgazi, Dinajpur Sadar,
23	Ali Hossain	Chehelgazi, Dinajpur Sadar,
24	Rozob Ali	Chehelgazi, Dinajpur Sadar,
25	Kasem Mia	Chehelgazi, Dinajpur Sadar,
26	Abdul Makek	Chehelgazi, Dinajpur Sadar,
27	Md. Khalek Bapari	Chehelgazi, Dinajpur Sadar,
28	Abdul Mozid	Chehelgazi, Dinajpur Sadar,
29	Zobbar Ali	Chehelgazi, Dinajpur Sadar,
30	Mannan Mia	Chehelgazi, Dinajpur Sadar,
31	Md. Alim Pattadar	Ishan Gopalpur, Faridpur Sadar
32	Md. Abdul Kuddus Mollah	Ishan Gopalpur, Faridpur Sadar
33	Md. Fazlu Bapari	Ishan Gopalpur, Faridpur Sadar
34	Md. Joynal Mridha	Ishan Gopalpur, Faridpur Sadar
35	Md. Munnaf Pattadar	Ishan Gopalpur, Faridpur Sadar

Appendix 1. Contd'

Sample No.	Farmer's Name	Address
36	Md. Siddique Mollah	Ishan Gopalpur, Faridpur Sadar
37	Md. Noznu Shek	Ishan Gopalpur, Faridpur Sadar
38	Md. Aziz Shikdar	Ishan Gopalpur, Faridpur Sadar
35	Md. Nurul Hauque	Ishan Gopalpur, Faridpur Sadar
40	Md. Siraz Hawlader	Ishan Gopalpur, Faridpur Sadar
41	Md. Kasem Ali	Debipur, Ishurdi, Pabna
42	Md. Zonbe Ali	Debipur, Ishurdi, Pabna
43	Md. Haidar Ali	Debipur, Ishurdi, Pabna
44	Md. Minhaz Uddin	Debipur, Ishurdi, Pabna
45	Md. Monzu Bishwas	Debipur, Ishurdi, Pabna
46	Md. Saiful Islam	Debipur, Ishurdi, Pabna
47	Md. Hakim	Debipur, Ishurdi, Pabna
48	Md. Abdul Malek	Debipur, Ishurdi, Pabna
49	Md. Moshlem Uddin	Debipur, Ishurdi, Pabna
50	Md. Ruhul Amin	Debipur, Ishurdi, Pabna
51	Ansar Ali	Fatehpur, Jhikargasa, Jessore
52	Md. Abdul Mozid	Fatehpur, Jhikargasa, Jessore
53	Rafiqul Islam	Fatehpur, Jhikargasa, Jessore
54	Ali Ershad	Fatehpur, Jhikargasa, Jessore
55	Abu Taleb	Fatehpur, Jhikargasa, Jessore
56	Joynal Abedin	Fatehpur, Jhikargasa, Jessore
57	Md. Rizaul	Fatehpur, Jhikargasa, Jessore
58	Mizanur Rahman	Fatehpur, Jhikargasa, Jessore
59	Abdul Mazid	Fatehpur, Jhikargasa, Jessore
60	Abdul Kader	Fatehpur, Jhikargasa, Jessore
61	Md. Alauddin	Durgapur, Ulipur, Kurigram
62	Md. Kamrul	Durgapur, Ulipur, Kurigram
63	Md. Abdus Samad	Durgapur, Ulipur, Kurigram
64	Md. Haider Ali Sardar	Durgapur, Ulipur, Kurigram
65	Md. Asek Mia	Durgapur, Ulipur, Kurigram
66	Md. Rafikuzzan Khokon	Durgapur, Ulipur, Kurigram
67	Md. Abdur Rashid	Durgapur, Ulipur, Kurigram
68	Rafiq Mia	Durgapur, Ulipur, Kurigram
69	Md. Abdus Hasen	Durgapur, Ulipur, Kurigram
70	Md. Awaluddin	Durgapur, Ulipur, Kurigram

Appendix 1. Contd'

Sample No.	Farmer's Name	Address
71	Md. Sukur Mia	Sutiakhali, Mymensingh Sadar
72	Md. Iman Ali	Sutiakhali, Mymensingh Sadar
73	Md. Azhar Ali	Sutiakhali, Mymensingh Sadar
74	Md. Idis Ali	Sutiakhali, Mymensingh Sadar
75	Md. Harun Mia	Sutiakhali, Mymensingh Sadar
76	Babor Ali	Sutiakhali, Mymensingh Sadar
77	Md. Belal Hossain	Sutiakhali, Mymensingh Sadar
78	Bazlu Mia	Sutiakhali, Mymensingh Sadar
79	Md. Mokaram	Sutiakhali, Mymensingh Sadar
80	Keramat Ali	Sutiakhali, Mymensingh Sadar
81	Md. Hafizur Rahman	Koyrabari, Atghoria, Pabna
82	Md. Ahad Ali Malitha	Koyrabari, Atghoria, Pabna
83	Md. Mirazdewan	Koyrabari, Atghoria, Pabna
84	Md. Hamid Sardae	Koyrabari, Atghoria, Pabna
85	Md. Rezaul Karim	Koyrabari, Atghoria, Pabna
86	Md. Sirazul Islam Sardae	Koyrabari, Atghoria, Pabna
87	Md. Jalal Uddin Pramanik	Koyrabari, Atghoria, Pabna
88	Md. Golap Malitha	Koyrabari, Atghoria, Pabna
89	Md. Kamrul Hasan	Koyrabari, Atghoria, Pabna
90	Md. Haidar Ali Sardae	Koyrabari, Atghoria, Pabna
91	Md. Akher Ali Pramanik	Aladipur, Razbari Sadar
92	Md. Belayet Mondol	Aladipur, Razbari Sadar
93	Md. Saker Ali Pramanik	Aladipur, Razbari Sadar
94	Md. Sams Mollah	Aladipur, Razbari Sadar
95	Md. Ishak Shekh	Aladipur, Razbari Sadar
96	Md. Sah Alam	Aladipur, Razbari Sadar
97	Md. Abu Taleb	Aladipur, Razbari Sadar
98	Md. Arshad Mollah	Aladipur, Razbari Sadar
99	Md. Sahjahan Khan	Aladipur, Razbari Sadar
100	Md. Bahadur Khan	Aladipur, Razbari Sadar

Appendix 2. Fungi detected in wheat seeds (cv. Kanchan) collected from ten different locations of Bangladesh

Sl. No.	Fungi detected in	
	Unclean seed	Clean seed
1	*Alternaria tenuis*	*Alternaria tenuis*
2	*Alternaria longissima*	–
3	*Aspergillus flavus*	*Aspergillus flavus*
4	*Aspergillus niger*	*Aspergillus niger*
5	*Bipolaris sorokiniana*	*Bipolaris sorokiniana*
6	*Chaetomium globosum*	*Chaetomium globosum*
7	*Cladosporium cladosporoides*	–
8	*Curvularia lunata*	*Curvularia lunata*
9	*Fusarium oxysporum*	*Fusarium oxysporum*
10	*Penicellium* sp.	*Penicellium* sp.
11	*Phoma* sp.	–
12	*Rhizopus nigricans*	*Rhizopus nigricans*
13	*Trichothecium roseum*	*Trichothecium roseum*