PREVALENCE OF FUNGI ASSOCIATED WITH SEVEN WHEAT VARIETIES AND SEED QUALITY ANALYSIS

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Abstract

Seven varieties of wheat seeds, such as BARI Gom-25, BARI Gom-26, BARI Gom-27, BARI Gom-28, BARI Gom-29, BARI Gom-30 and Kanchan were studied to determine the prevalence of seed-borne fungi and their effects on germinating seeds and seedlings. The seeds were categorized into apparently healthy seeds, black point seeds, and shriveled and undersized seeds. Variation in different parameters (viz.Grading, weight, purity percentage, germination and seedling growth) became evident among the varieties. Seed health test on blotters and agar plate method showed that the major fungi associated with healthy seeds and black point seeds were *Alternaria alternata*, *A. triticina*, *Bipolaris sorokiniana*, *B. tetramera*, *Curvularia lunata*, *Fusarium* spp., and species of *Aspergillus*. The incidence of *B. sorokiniana* increased with increasing severity of black point infection. Reduction in germination, seedling emergence, plant stand, root and shoot growth, and vigor index were directly related with the severity of black point. *Aspergillus* spp. were seen more frequently associated with non-germinated seeds.

Key words: Black point; Seed quality; Seed-borne fungi; Wheat.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is considered as the second most staple food crop next to rice in Bangladesh. But the average yield of wheat in Bangladesh is lower in comparison to other countries. During 2018-19, wheat production was 1.02 million metric tons from 0.3 million ha (BBS 2019). Diseases play an important role in lowering wheat yield in the country. Wheat is attacked by at least 20 different diseases in Bangladesh including twelve seed transmitted. All these seed-borne diseases are caused by fungi (Fakir *et al.* 1977, Fakir 1980, Ahmed and Hossain 1985, Ahmed 1986, Alam *et al.* 1994).

Seeds are the carrier of various plant pathogenic fungi, bacteria, viruses, nematodes and even angiospermic parasites. In most of the cases, infected seeds produce a range of symptoms on the seeds. These symptoms are seed rot, shriveling of grain, seed necrosis and seed discoloration. According to Neergaard (1979), noticeable brown, grey or black necrotic stain on the seed coat of many seeds is caused by the infection of many parasitic seed-borne fungi. Brown to dark brown or black discoloration mainly limited to the embryonic area of wheat seed is recognized as black point. Sometimes, the discoloration can be seen near the brush end, in the ventral crease, or on any other part of the grain. When the discoloration affects more than one-half of the grain it is interpreted as kernel smudge. In severe infection the whole grain may be discolored, shriveled and undersized. The discoloration may be light to dark and fairly uniform in color or it may appear as light colored lesions with dark margins. Black point typically not yields reducing, but decreases grade and quality of grain. This type of seed discoloration is caused primarily by *Bipolaris sorokiniana* and *Alternaria alternata* (Fakir *et al.*1989, Dey *et al.* 1992, Mathur and Cunfer 1993). A large number of fungal floras were reported with black point seeds in Bangladesh (Fakir *et al.* 1987, Islam *et al.* 2015). Malaker and Mian (2002) detected *B. sorokiniana*, *A. alternata*, *Curvularia lunata* and *Fusarium* spp. and observed that the incidence of *B. sorokiniana* increased with the increase in severity of black point infection. Malaker *et al.* (2007) identified 22 fungi representing 17 genera from black point infected seeds collected from the major wheat growing areas of Bangladesh. The most predominant fungus was *B. sorokiniana*, which was followed by *A. alternata* and *C. lunata*. Among the fungi associated with the black point disease of wheat seeds in Bangladesh, *B. sorokiniana*

(Sacc.) Shoemaker (syn. *Helminthosporium sativum*, *Drechslera sorokiniana*) appeared to be the most predominant one, which also causes seed rot or germination failure, seedling blight, leaf blight and head blight in wheat (Hossain 2000).

In Bangladesh, the black point disease of wheat occurs in almost all wheat growing areas with varying degrees of severity. The prevalence of the disease was found to vary from 2-15% in some selected commercial wheat cultivars (Ahmed 1986). Rahman and Islam (1998) recorded 4-14% black point affected seed with respect to seed tier, variety and seed source. Dey *et al.* (1992) reported 5-55% black point infection depending on different varieties grown in the major wheat areas of the country. The adverse effects of black point on seed germination and seedling vigor have been documented by many workers (Malakar and Mian 2002, Siddique *et al.* 2002, Rashid *et al.* 2004). The emergence of the root and shoot growth of seedlings decreased with the increase in incidence and severity of black point infection. Plant stand and grain yield were also reduced when black point affected seeds were used for sowing (Malakar and Mian 2002). The grain weight of wheat was reported to be reduced by 42% when the grains were severely infected with the black point disease (Rahman and Islam 1998). The present research project was undertaken with the objectives to seed quality analysis with the prevalence of seed-borne fungi in wheat and their effect on germinating seed and seedlings.

MATERIAL AND METHODS

Collection of wheat seed samples

Freshly harvested wheat seeds were collected from seven varieties in the year 2015. Out of them, six samples were taken from six varieties (viz. BARI Gom-25, BARI Gom-26, BARI Gom-27, BARI Gom-28, BARI Gom-29, and BARI Gom-30) from Bangladesh Agricultural Research Institute (BARI). From a single variety named 'Kanchan' two samples were taken (one from BARI and another from Dhaka University campus) in the investigation. The total samples were eight in number. The size of each seed sample was 500 g (approx.). The seeds were then kept in brown paper bags covered with polythene and stored in the refrigerator at 5-7^{o}C, till these were used for subsequent studies.

Preparation of working sample

Four hundred seeds from each sample were taken randomly for seed quality/seed health test, that is sorting of apparently healthy seed (best seed), black point seed and others, detection of seed borne fungal pathogens for germination, seed viability, mortality and seedling vigour etc.

$$\text{Purity Percentage of seed (\%)} = \frac{Weight\ of\ pure\ seeds\ (g)}{Total\ Weight\ of\ seeds\ (g)} \times 100$$

Black point incidence of wheat

Black point seeds were sorted from the collected seed samples manually by eye estimation.

$$\text{Percent black point incidence} = \frac{\text{Total no.of black point seeds}}{\text{Total number of seeds examined}} \times 100$$

Severity of the black point infection of wheat

The seeds were sorted out into 6 different grades on the basis of severity of black point infection. The grading was done according to a 0-5 rating scale as suggested by Gilchrist (1985), where

Grade 0 = Grains free from any discoloration (apparently healthy),
Grade 1 = Only tip of the embryo brown to blackish,
Grade 2 = Discoloration covering the whole embryo,
Grade 3 = Embryo with $^1/_4$ of the grain discolored,
Grade 4 = Embryo with $^1/_2$ of the grain discolored and
Grade 5 = Embryo with more than $^1/_2$ of the grain discolored and shriveled.

Isolation, Purification and identification of fungi

The International Seed Testing Association (ISTA 1996) as an internationally recognized organization providing standardized methods for routine seed quality testing. There is a number of methods of seed health testing (ISTA-SHT) depending on the nature of the pathogen and on the type of seed. Standard Blotter method (Limonard 1966) and PDA method (Malone and Muskett 1964) as suggested by ISTA were used for the detection of seed-borne mycoflora.

The Blotter method

Four hundred seeds were randomly taken from each of the seed samples and sorted into healthy and black pointed seeds. Twenty-five healthy seeds (except 10 seeds in case of black point) are placed on three layers of water soaked filter papers contained in each of 9 cm Petri plate. The Petri plates were incubated usually for 7 days under 12 hours alternating cycles of light and darkness. After incubation, fungi developed on the seeds were examined under different magnification of a stereomicroscope and identified. The identification of the fungi is based on the way they grow on the seeds and on the morphological characters of fruiting bodies, spores/conidia observed under a compound microscope. The seeds were surface sterilized in 10% Clorox for three minutes and then rinsed for two minutes each in three changes of sterile distilled water prior to plating.

The Agar plate method

The agar plate method is another popular method in which ten seeds (both healthy and black point seeds) were plated in each Petri plate containing 15 ml of potato dextrose agar (PDA) medium and incubated for 5-7 days at 25±2°C under 12 hours alternating cycles of light and darkness. At the end of the incubation period, fungi growing out from seeds on the medium were examined and identified. Identification was based on colony characters and morphology of sporulating structures under a compound microscope. The photomicrographs of fungal colonies and spores were taken. The generic and species identity of each colony was recorded and identification was determined following standard literatures (Barnett and Hunter 1972, Benoit and Mathur 1970, Booth 1971, Chidambaram *et al.* 1973, Ellis 1971, 1976, Ellis and Ellis 1997, Raper and Thom 1949, Thom and Raper 1945, Subramanian 1971, Sutton 1980).

Percentage frequency of the occurrence of the fungal isolates was calculated by adopting the following formula (Spurr and Welty 1972):

$$\text{Frequency (\%)} = \frac{\text{No.of inocula from which fungal isolates were raised}}{\text{total number of inocula culture}} \times 100$$

The Seedling germination test

For germination test fifty seeds were sown in each plastic tray filled with sterilized sandy soil. Care was taken to ensure the proper sunlight and moisture in the soil of the tray for 14 days. Then

data on seed germination percentage, mortality percentage were recorded according to International Seed Testing Association Rules (ISTA 1996). Seeds producing both plumule and radical were considered as germinated seeds.

The germinated seeds were counted and expressed as a measure of seed viability (*Sv*) using the formula.

$$\text{Seed viability,} \quad Sv = \frac{n}{N} \times 100$$

Where, *Sv* = % seed viability; *n* = the number of seeds germinated from each normal or abnormal seed type; and *N* = the total number of seeds used

Measurement of plant shoots, and root growth and weight

The soil in the tray was first irrigated to make it moist so that uprooting of the emerged plant would be simple. The plant and root were then removed from the dish and submerged in a pail of water. Each seedling's root was gently cleansed and rubbed clean under running water. With a sharp knife, the root and shoot portions were separated. From each tray, ten (10) seedlings were chosen at random, and each one's unique shoot length was measured from the stem's base to the point where the youngest leaf was growing, the length of the root was measured from its starting point to the largest available lateral root apex. With the use of a digital balance, the fresh weight of the shoot and root was also assessed.

Seed vigor, an important index of seed quality, determines the potential for rapid and uniform emergence of plants. Planting low vigor seed caused reduced plant height, delayed panicle exertion, and anthesis, less tillering capacity, and reduced yield. The vigor index (VI) of the seedlings can be estimated as suggested by Abdul-Baki and Anderson (1973):

$$VI = RL + SL \times GP$$

Where, RL= Root length (cm); SL= Shoot length (cm); and GP= Germination percentage.

RESULTS AND DISCUSSION

Increased crop productivity can be achieved by using the cultivars of high-yielding varieties and avoiding crop failures. This involves the demand of better quality seed in terms of germination, purity and health status. Every year, about 20% of wheat loss is reported due to disease which would be available for food and feed (Fakir 1999).

Seed quality analysis

Seeds were classified into three classes- apparently healthy seeds, black point seeds and shriveled and undersized seeds (Fig. 1). The moisture contents of the seed samples varied from 13.4-13.8.

Apparently healthy seeds

Quality analysis showed that the percentage of pure seeds/apparently healthy seeds varied from 85.02% - 93.06% (Table 1). The highest percentage of apparently healthy seeds was recorded in BARI Gom-30 followed by BARI Gom-25 (90.71%), BARI Gom-29 (90.56%) and BARI Gom-27 (90.06%). The lowest percentage of apparently healthy seeds was found in Kanchan (DU) variety.

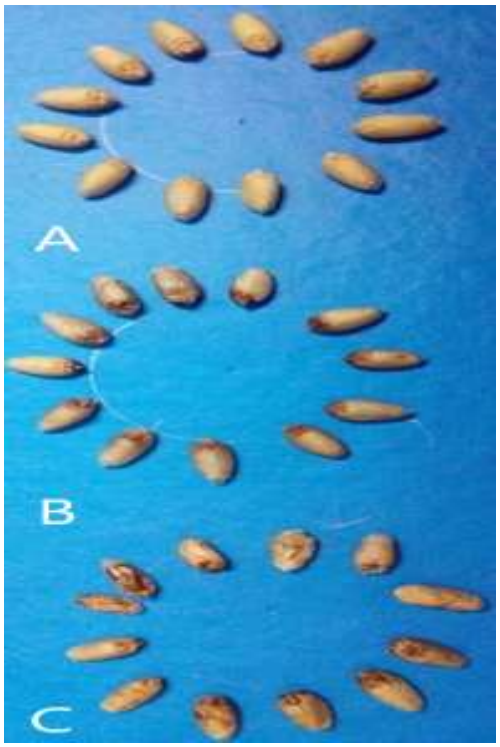

Fig. 1. A. Pure seeds/apparently healthy seeds
B. Black point seeds C. Shriveled & undersized seeds.

Fig. 2. G_0= Grains free from discoloration (apparently healthy), G_1= Only tip of the embryo brown to blackish, G_2= Discoloration covering the whole embryo, G_3= Embryo with $^1/_4$ of the grain discolored, G_4= Embryo with $^1/_2$ of the grain discolored, G_5= Embryo with more than $^1/_2$ of the grain discolored and shriveled.

Black point seeds

Table 1. shows that the highest amount of black point seeds (8.72%) was recorded in BARI Gom-28 followed by BARI Gom-29 (8.36%) and the lowest (4.04%) found in Kanchan (BARI) variety.

Table 1. Percentage of apparently healthy seeds, black point seeds and shriveled and undersized seeds with moisture contents.

Sl. No.	Name of wheat varieties	Apparently healthy seeds (%)	Black point seeds (%)	Shriveled & undersized seeds (%)	Moisture contents (%)
1	BARI Gom-25	90.71	6.56	2.74	13.5
2	BARI Gom-26	89.19	7.46	3.34	13.4
3	BARI Gom-27	90.06	4.23	5.71	13.6
4	BARI Gom-28	89.74	8.72	1.54	13.4
5	BARI Gom-29	90.56	8.36	1.08	13.5
6	BARI Gom-30	93.06	4.93	2.0	13.3
7	Kanchan (BARI)	87.26	4.04	8.7	13.5
8	Kanchan (DU)	85.02	7.84	7.14	13.8

Shriveled and undersized seeds

The percentage of shriveled and undersized seeds varies from 1.08 to 8.7%. The highest occurrence of shriveled and undersized seeds (8.7%) was recorded in Kanchan (BARI) variety followed by Kanchan (DU) (7.14%), and the lowest was (1.08%) in BARI Gom-29.

Grading of wheat seeds

According to Gilchrist (1985) the wheat seeds of seven varieties were categorized from G_0 to G_5 grade on the basis of percentage (Table 2 and Fig. 2). The G_0 means that the grains were free from discoloration and these were apparently healthy seeds; so, the data were the same presented in Table 1. The G_1 means that only tip of the embryo was brown to blackish; the highest percentage of G_1 grade seeds was recorded in BARI Gom-28 (2.56%). The maximum G_2 grade seeds were also recorded in BARI Gom-28 (2.74%); the G_3 grade seeds were the highest in BARI Gom-29 (2.71%); the G_4 grade was in Kanchan (DU) (2.48%); the G_5 means embryo with more than $^1/_2$ of the grain discolored and shriveled, and the maximum G_5 grade was also in Kanchan (DU) (7.14%).

Table 2. Percentage of G_0-G_5 grades of wheat seeds on the basis of severity of black point infection.

Sl. No.	Name of wheat varieties	Grades of wheat grain (Percentage %)					
		G_0	G_1	G_2	G_3	G_4	G_5
1	BARI Gom-25	90.71	1.66	1.49	1.92	1.5	2.74
2	BARI Gom-26	89.19	1.97	1.72	1.89	1.98	3.34
3	BARI Gom-27	90.06	1.06	0.85	0.92	1.41	5.71
4	BARI Gom-28	89.74	2.56	2.74	2.31	1.12	1.54
5	BARI Gom-29	90.56	1.7	2.17	2.71	1.78	1.08
6	BARI Gom-30	93.06	1.17	1.5	1.0	1.25	2.0
7	Kanchan (BARI)	87.26	0.78	0.93	1.16	1.16	8.7
8	Kanchan (DU)	85.02	1.5	2.02	1.84	2.48	7.14

Purity percentage

The purity percentage of wheat seeds varied from 87.83 to 94.05% (Fig. 3). The highest purity percentage was in the variety BARI Gom-27 followed by the BARI Gom-30 (93.34%) and the BARI Gom-25 (92.25%); and the lowest percentage was in Kanchan (DU).

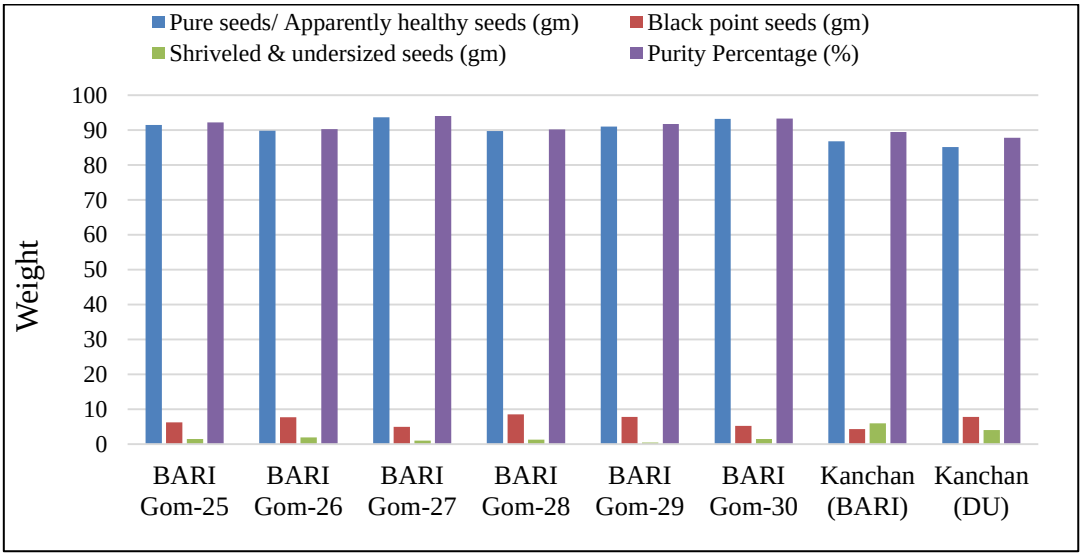

Fig. 3. Weight of apparently healthy seeds, black point seeds and shriveled & undersized seeds of seven wheat varieties (100 g) with purity percentage.

Another weight parameter was presented in Fig. 4, which shows 1000 seed weight (g) differences among three different types of seeds. The 1000 seed weight of apparently healthy seeds was the highest in BARI Gom-30 (63 g) followed by BARI Gom-28 (62 g) and the lowest was in variety

Kanchan (DU) (45 g). The maximum differences in 1000 seed weight between apparently healthy seeds and black point seeds were noticed in the variety BARI Gom-29 (57 g and 42 g) and BARI Gom-30 (63 g and 49 g). From the result we can understand how yield loss varied in the varieties due to black point disease. This depends on the percentage of black point seeds and shriveled & undersized seeds and also their weight reduction compares to healthy seeds.

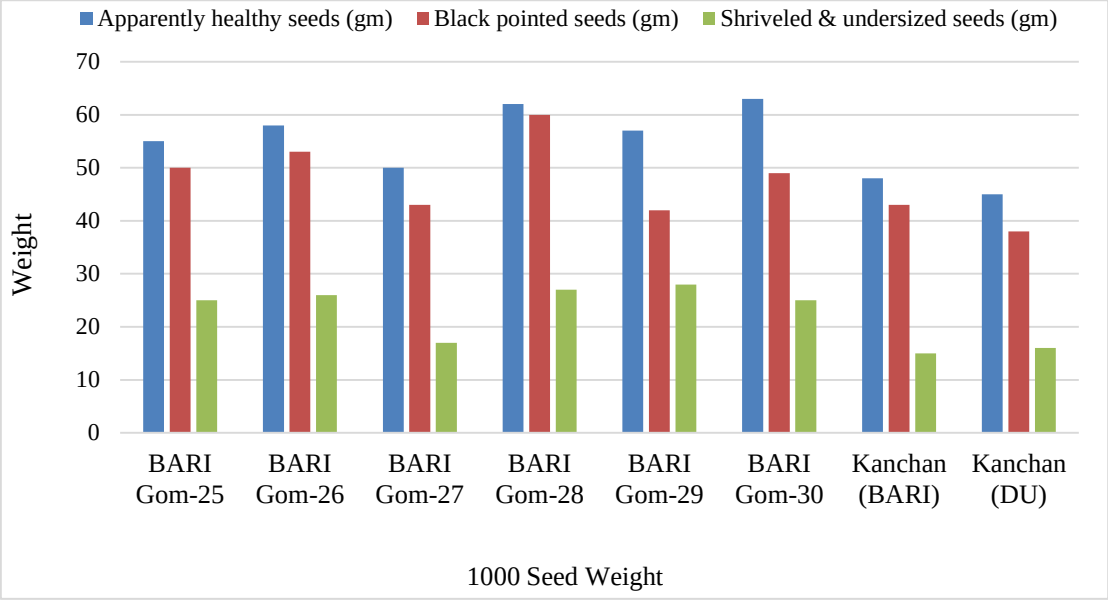

Fig. 4. 1000 seed weight of apparently healthy seeds, black point seeds and shriveled & undersized seeds of seven wheat varieties.

Determination of germination

It appears from the Table 3 and Fig. 5 that about 90% of germination was recorded for the seven wheat varieties after 7 days. The highest germination of healthy seed (95%) was recorded in the variety Kanchan (BARI) followed by the BARI Gom-27 (94.67%) and the BARI Gom-25 (93.67%). The differences of germination percentage between apparently healthy seeds and black point seeds were up to 10%. The percentage of seedling emergence varied in apparently healthy seeds 75-91%, where as in case of black point seeds the range was 62.34-72%. As a result, mortality percentage was higher in the black point seeds (10.48-21.09%) compared to the apparently healthy seeds (3.37-16.73%). The maximum seedling emergence percentage in the apparently healthy seeds was achieved by the BARI Gom-27 (91%) followed by the Kanchan (BARI) (90%). Though variety Kanchan was used in this experiment as a susceptible check, but germination and seedling emergence quality was better than other advanced variety. The variation in the germination percentage may be occurred due to variation in genetic makeup or the association of seed borne pathogens. The prevalence of seed-borne infection is also responsible for lower germination (Fakir 1998, Islam *et al*. 2015). Hossain (2000) observed that black point infection greatly affected seed germination and seedling emergence of wheat, and percentage reduction in germination becomes higher with the increasing level of black pointed seed. The germination of seed infected with seed-borne pathogens may cause pathogens to attack the embryo and eventually kill the seedlings. Seed-borne diseases have been found to affect the growth and productivity of crop plants.

Table 3. Effect of apparently healthy seeds and black point seeds on seed viability, seedling emergence and mortality percentage.

Sl. No.	Name of wheat varieties	Germination percentage /Seed viability (%)		Percentage of seedling emergence (%)		Percentage of seedling mortality (%)	
		HS	BPS	HS	BPS	HS	BPS
1	BARI Gom-25	93.67	81.34	78	66.34	16.73	18.44
2	BARI Gom-26	90	79	75	62.34	16.67	21.09
3	BARI Gom-27	94.67	82	91	71.34	3.88	13
4	BARI Gom-28	89.0	76.34	86.0	68.34	3.37	10.48
5	BARI Gom-29	91	80.34	82.0	70	9.89	12.87
6	BARI Gom-30	92	81.34	86.34	70	6.15	13.94
7	Kanchan (BARI)	95	84	90	72	5.26	14.28
8	Kanchan (DU)	93	82	88.34	69	5.01	15.85

HS = Healthy seed, BPS = Black point seed

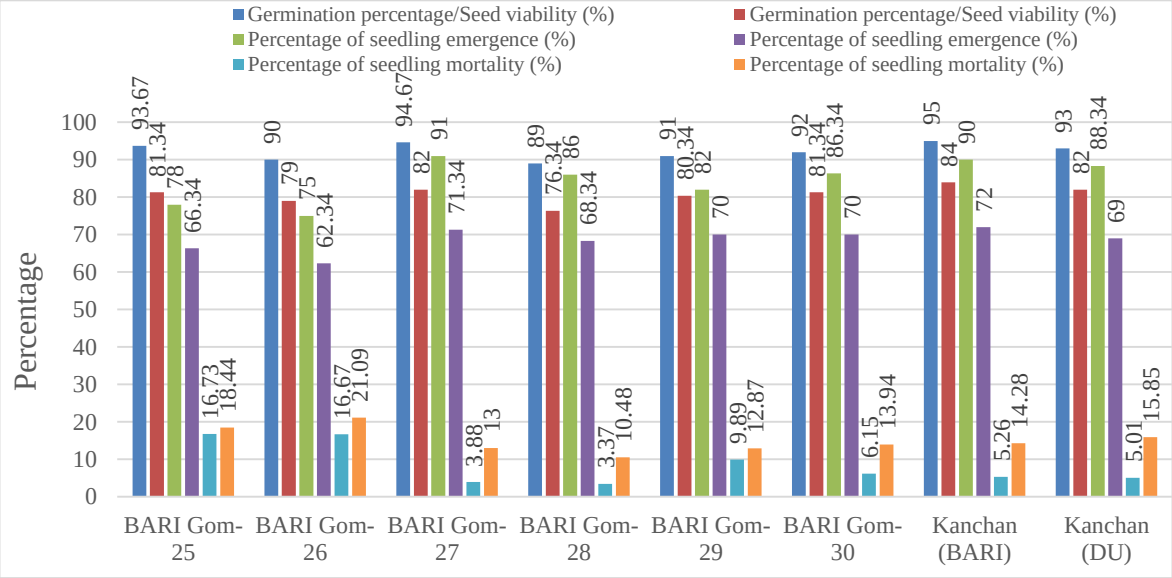

Fig. 5. Effects of apparently healthy seeds and black point seeds on seed viability, seedling emergence and mortality percentage.

Determination of vigor index

Vigor index was related to shoot length, root length and germination percentage. In apparently healthy seeds and black point seeds, the highest shoot length was recorded in the BARI Gom-25 (25.56 cm and 20.2 cm, respectively) followed by the BARI Gom-27 (24.82 cm and 16.8 cm, respectively) and the lowest shoot length was recorded in the Kanchan (DU) (15.13 cm and 8.46 cm, respectively) followed by the Kanchan (BARI) (15.62 cm and 8.62 cm, respectively). In case of root length, the apparently healthy seeds and black point seeds showed the maximum root length in the BARI Gom-27 (30.8 cm and 20.8 cm, respectively) followed by the BARI Gom-29 (18.06 cm and 14.02 cm, respectively) and minimum was in variety the Kanchan (DU) (9.96 cm in apparently healthy seeds) and the Kanchan (BARI) (7.72 cm in black point seeds). So, in both cases vigor index was best in the BARI Gom-27 (5265.54 and 3083.2, respectively) followed by the BARI Gom-25 (3836.72 and 2461.35, respectively) and the least vigor index was Kanchan (DU) (2333.37 and 1372.56, respectively) followed by the Kanchan (BARI) (2441.5 and 1372.56, respectively) (Table 4).

Table 4. Effects of apparently healthy seeds and black point seeds on seedling growth with vigor index.

Sl. No	Wheat variety	Shoot length (cm)		Root length (cm)		Fresh shoot weight (g)		Fresh root weight (g)		Vigor Index	
		HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS
1	BARI Gom-25	25.56	20.2	15.4	10.06	0.17	0.1	0.19	0.11	3836.72	2461.35
2	BARI Gom-26	21.56	15.32	12.92	9.44	0.17	0.11	0.48	0.31	3103.2	1956.04
3	BARI Gom-27	24.82	16.8	30.8	20.8	0.18	0.12	0.65	0.46	5265.54	3083.2
4	BARI Gom-28	21.32	12.96	10.06	9.2	0.16	0.1	0.16	0.1	2792.82	1691.69
5	BARI Gom-29	20	13.7	18.06	14.02	0.18	0.12	0.18	0.11	3463.46	2227.02
6	BARI Gom-30	20.36	15.2	12.62	9.04	0.17	0.11	0.30	0.21	3034.16	1971.68
7	Kanchan (BARI)	15.62	8.62	10.08	7.72	0.06	0.04	0.07	0.05	2441.5	1372.56
8	Kanchan (DU)	15.13	8.46	9.96	7.92	0.06	0.04	0.07	0.05	2333.37	1343.16

HS = Healthy seed, BPS = Black point seed

Again fresh shoot weight and fresh root weight were also measured in apparently healthy seeds and black point seeds. Differences were noticed not only between apparently healthy seeds and black point seeds, but also susceptible variety Kanchan with other high yielding wheat varieties. Seed vigor is not equivalent of seed germinability (Fig. 6 A and B). According to ISTA, vigor is expressed as the sum total of those properties of seed which determine the potential level of activities and performance of a non-dormant seed (Perry 1972), thus germination tests can only point out the capability of a seed lot, established seedling which are conducted under optimum condition in the research laboratory. A vigor test evaluates, either directly or indirectly, the physiological and physical basis of potential seed lot performance and offers a more sensitive distinction among seed lots than does the standard germination test. Even if seed vigor tests are generally used by the seed industry and are now offered by the two-thirds of ISTA seed testing laboratories.

Fig. 6. A. Shoot of ten healthy seeds. B. Shoot of ten black point seeds.

Detection of seed borne fungi associated with wheat seeds

The Blotter Method

The results of present study revealed that the wheat seeds are quite frequently infected by fungi. A total of twelve genera, i.e. *Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Curvularia* spp., *Chaetomium* sp., *Cladosporium* sp., *Drechslera* sp., *Epicoccum* sp., *Fusarium* spp., *Nigrospora* sp., *Penicillium* sp. and *Rhizopus* sp. was isolated from seven wheat varieties (Table 5). *Alternaria alternata* and *A. triticina* showed the highest (26.1% and 13.05%, respectively) infections on the Kanchan (DU). *Bipolaris sorokiniana* was the highest (30.45%) in the Kanchan (DU) followed by the Kanchan (BARI) (26.3%) and the BARI Gom-27 (22.73%). Variation in the association of *Aspergillus* spp. with different wheat varieties was noticed (Table 5). Sulaiman and Hussain (1984)

observed that *Aspergillus flavus* reduced 90% germination of wheat seeds as compared to healthy seeds. *Bipolaris oryzae* was obtained from only BARI Gom-30 in blotter method, but *Bipolaris tetramera* was isolated frequently. The highest 26.09% in *Curvularia lunata* and 21.74% in *Fusarium* spp. were found in the Kanchan (DU) variety (Fig 7 A, B).

Table 5. Frequency percentage of fungi from apparently healthy seeds and black point seeds of wheat detected by Blotter Method.

Sl. No.	Name of Fungi	BARI Gom-25		BARI Gom-26		BARI Gom-27		BARI Gom-28		BARI Gom-29		BARI Gom-30		Kanchan (BARI)		Kanchan (DU)	
		HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS
1	*Alternaria alternata*	6.25	14.58	5.4	13.51	4.54	18.18	4.16	16.67	9.09	18.18	9.52	19.05	-	10.53	4.35	26.1
2	*Alternaria triticina*	2.08	6.25	-	5.4	-	9.09	-	-	-	4.54	-	-	5.26	5.26	-	13.05
3	*Aspergillus flavus*	4.17	8.34	-	2.7	4.53	13.64	-	8.33	9.09	22.73	4.76	14.28	-	10.53	4.35	8.69
4	*Aspergillus fumigatus*	6.25	6.25	2.7	8.12	4.54	18.18	8.33	16.67	9.09	22.73	9.52	28.57	-	-	-	-
5	*Aspergillus niger*	16.7	33.34	5.4	21.62	-	4.54	-	8.33	-	9.09	-	4.76	10.5	10.53	-	8.69
6	*Aspergillus terrous*	10.4	22.92	8.12	8.12	-	-	-	-	-	-	-	-	-	5.26	-	-
7	*Bipolaris oryzae*	-	-	-	-	-	-	-	-	-	-	-	4.76	-	-	-	-
8	*Bipolaris sorokiniana*	-	8.34	5.4	10.81	9.09	22.73	-	12.5	4.54	13.64	4.76	19.05	10.5	26.3	8.69	30.45
9	*Bipolaris tetramera*	2.08	6.25	2.7	13.51	-	4.54	-	8.33	-	-	-	9.52	10.5	21.05	8.69	17.39
10	*Chaetomium* sp.	10.4	12.5	-	8.12	-	4.54	-	-	9.09	9.09	-	9.52	-	-	-	-
11	*Cladosporium* sp.	-	-	-	-	-	-	8.33	-	-	-	-	-	-	-	4.35	-
12	*Curvularia lunata*	8.34	8.34	2.7	13.51	4.54	9.09	-	12.5	-	9.09	4.46	9.52	5.26	15.79	13	26.09
13	*Curvularia pallescens*	-	2.08	-	8.12	-	-	-	-	-	-	-	4.46	-	-	4.35	8.69
14	*Curvularia* sp.	-	-	-	-	-	4.54	-	4.16	-	-	-	-	-	-	-	-
15	*Drechslera* sp.	-	-	-	-	-	-	12.5	-	-	-	-	-	-	-	4.35	-
16	*Epicoccum* sp.	-	4.17	-	-	-	-	-	-	-	2.27	-	-	-	-	-	-
17	*Fusarium* sp.	2.08	8.34	-	8.12	-	9.09	4.16	4.16	9.09	9.09	4.46	9.52	10.5	26.31	13	21.74
18	*Nigrospora* sp.	-	2.08	-	-	-	-	-	-	4.54	9.09	-	-	-	-	-	4.35
19	*Penicillum* sp.	-	-	-	8.12	-	-	-	8.33	-	-	4.46	4.46	-	-	4.35	-
20	*Rhizopus* sp.	-	2.08	-	-	-	9.09	-	-	-	-	-	9.52	-	-	-	-
21	*Trichoderma* sp.	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

HS = Healthy seed, BPS = Black point seed

The percentage of fungal infection of wheat varieties ranged from 2.08 to 30.45%. The species *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Bipolaris sorokiniana*, *Curvularia lunata* and *Fusarium* spp. were: isolated from seven varieties of wheat seeds. The incidences of *Aspergillus fumigatus*, *A. terrous*, *Cheatomium* sp., *Cladosporium* sp., *Curvularia pallescens*, *Nigrospora* sp., *Penicillium* sp., were also remarkable from several varieties of wheat seeds.

In total more than twenty species belonging to twelve genera of fungi were indentified; the most pre-dominant fungi were *Alternaria alternata*, *Aspergillus flavus*, *A. niger*, *Bipolaris sorokiniana*,

Curvularia lunata and *Fusarium* spp. Naznine *et al.* (2016) conducted a seed health test by following the blotter method which resulted in seven different seed borne fungi species belonging to six genera, *viz. Bipolaris sorokiniana* (0.5-30.5%), *Alternaria tenuis* (0.5-25%), *Fusarium moniliforme* (0.0-33.5%), *Fusarium oxysporum* (2.7-53%), *Curvularia lunata* (0.0-5.5%), *Aspergillus niger* (0.0-18.5%) and *Penicillium* sp. (0.0-1.5%). Out of 11samples tested the germination of seeds ranged from 98.0 to 73.5%. Their findings are almost similar to the present investigation.

Agar Plate Method

By following the agar plate method, a total of thirteen genera, i.e. *Alternaria* spp., *Aspergillus* spp., *Bipolaris* spp., *Curvularia* spp., *Chaetomium* sp., *Cladosporium* sp., *Drechslera* sp., *Epicoccum* sp., *Fusarium* spp., *Nigrospora* sp., *Penicillium* sp., *Rhizopus* sp. and *Trichoderma* spp. was recorded (Table 6).

Alternaria alternata showed the highest (27.24%) fungal infections on the the Kanchan (DU) followed by the BARI Gom-27 (21.05%) on black point seeds. But, *Alternaria triticina* was the highest in the variety Kanchan (BARI) (23.8%) followed by the BARI Gom-29 (13.64%) on black point seeds. *Bipolaris sorokiniana* was isolated from all the varieties; this species was found the highest (45.45%) in the BARI Gom-29 followed by 42.86% in the BARI Gom-28 on black point seeds. Frequency percentage of *Aspergillus fumigatus* was the highest on the BARI Gom-28 (28.57%) followed by the Kanchan (DU) (27.24%) on black point seeds. The highest (11.76%) *Curvularia lunata* was found in the BARI Gom-26 variety and *Fusarium* spp. was the highest on the Kanchan (DU) (13.62%) on black point seeds (Fig 7 C, D).

Table 6. Frequency percentage of fungi from apparently healthy seeds and black point seeds of wheat detected by Agar Plate Method.

Sl. No.	Name of Fungi	BARI Gom-25		BARI Gom-26		BARI Gom-27		BARI Gom-28		BARI Gom-29		BARI Gom-30		Kanchan (BARI)		Kanchan (DU)	
		HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS	HS	BPS
1	*Alternaria alternata*	6.25	18.75	5.88	11.76	5.26	21.05	4.76	14.28	9.09	13.64	5.88	11.76	4.76	14.28	9.09	27.24
2	*Alternaria triticina*	-	-	-	5.88	-	-	-	-	4.54	13.64	-	5.88	4.76	23.8	4.54	9.09
3	*Aspergillus flavus*	-	18.75	-	5.88	5.26	-	4.76	-	-	-	5.88	-	9.52	-	-	9.09
4	*Aspergillus fumigates*	-	25	5.88	23.53	-	-	4.76	28.57	9.09	22.73	-	11.76	-	23.8	-	27.24
5	*Aspergillus niger*	6.25	-	-	5.88	-	10.53	-	9.52	-	4.54	-	-	-	-	4.54	13.62
6	*Aspergillus terrous*	-	-	-	-	5.26	-	-	-	4.54	-	-	-	-	-	-	4.54
7	*Bipolaris oryzae*	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	*Bipolaris sorokiniana*	6.25	18.75	5.88	17.65		5.26	9.52	42.86	9.09	45.45	11.76	11.76	9.52	33.32	4.45	22.25
9	*Bipolaris tetramera*	-	-	-	-	5.26	-	-	-	-	-	-	11.76	-	4.76	-	9.09
10	*Chaetomium* sp.	-	-	-	-	-	-	-	-	-	-	-	11.76	-	-	-	-
11	*Cladosporium* sp.	6.25	-	-	-	-	-	-	-	-	-	-	-	9.52	-	-	-
12	*Curvularia lunata*	-	6.25	-	11.76	-	5.25	-	9.52	4.54	-	5.88	17.65	9.52	9.52	-	9.09
13	*Curvularia pallescens*	-	-	-	-	-	5.26	-	-	-	9.09	5.88	5.88	-	-	-	-
14	*Curvularia* sp.	-	-	5.88	-	-	-	4.76	-	-	-	-	-	-	-	-	-
15	*Drechslera* sp.	-	-	-	-	-	21.05	-	-	-	-	-	-	-	-	14.28	-
16	*Epicoccum* sp.	-	6.25	-	-	-	-	-	-	-	-	-	2.94	2.38	-	-	-
17	*Fusarium* sp.	6.25	12.5	-	5.88	5.26	5.26	4.76	-	4.54	9.09	-	-	9.52	-	-	13.62
18	*Nigrospora*sp.	-	6.25	-	-	-	-	-	-	-	9.09	-	-	-	9.52	-	-
19	*Penicillum* sp.	6.25	-	-	-	-	5.26	-	-	-	-	-	-	-	-	-	-
20	*Rhizopus* sp.	-	-	-	5.88	-	-	-	-	-	-	-	-	4.76	-	-	-
21	*Trichoderma* sp.	-	-	5.88	-	-	-	-	-	-	-	-	-	-	-	-	4.76

HS = Healthy seed, BPS = Black point seed

The frequency percentage of fungal infection on different wheat varieties ranged from 2.38% to 45.45%. The species *Alternaria alternata*, *Bipolaris sorokiniana*, *Curvularia lunata* and *Fusarium* spp. were isolated from seven varieties of wheat seeds. The incidence of *Alternaria triticina*,

Aspergillus flavus, *A. fumigatus*, *A. niger* and *Curvularia lunata* was notable. In total twenty-one species of fungi were identified; the most pre-dominant fungi were the species of *Alternaria* spp., *Aspergillus* spp., *Bipolaris sorokiniana*, *Curvularia* spp. and *Fusarium* spp. A considerable number of seed-borne fungal pathogens belonging to the genera *Bipolaris*, *Alternaria*, *Curvularia*, *Fusarium*, *Penicillium* and *Aspergillus* was detected in wheat seeds as reported by many researchers (Ashrafuzzaman and Hossain 1992, Hossain and Schlosser 1993). Higher level of *Bipolaris* infected seed causes higher level of disease in adult plant (Malaker 2003). Leaf spot/leaf blight disease is the consequence of seed to plant and again to seed transmission of *B. sorokiniana* (Nema and Joshi 1974, Hossain 2000).

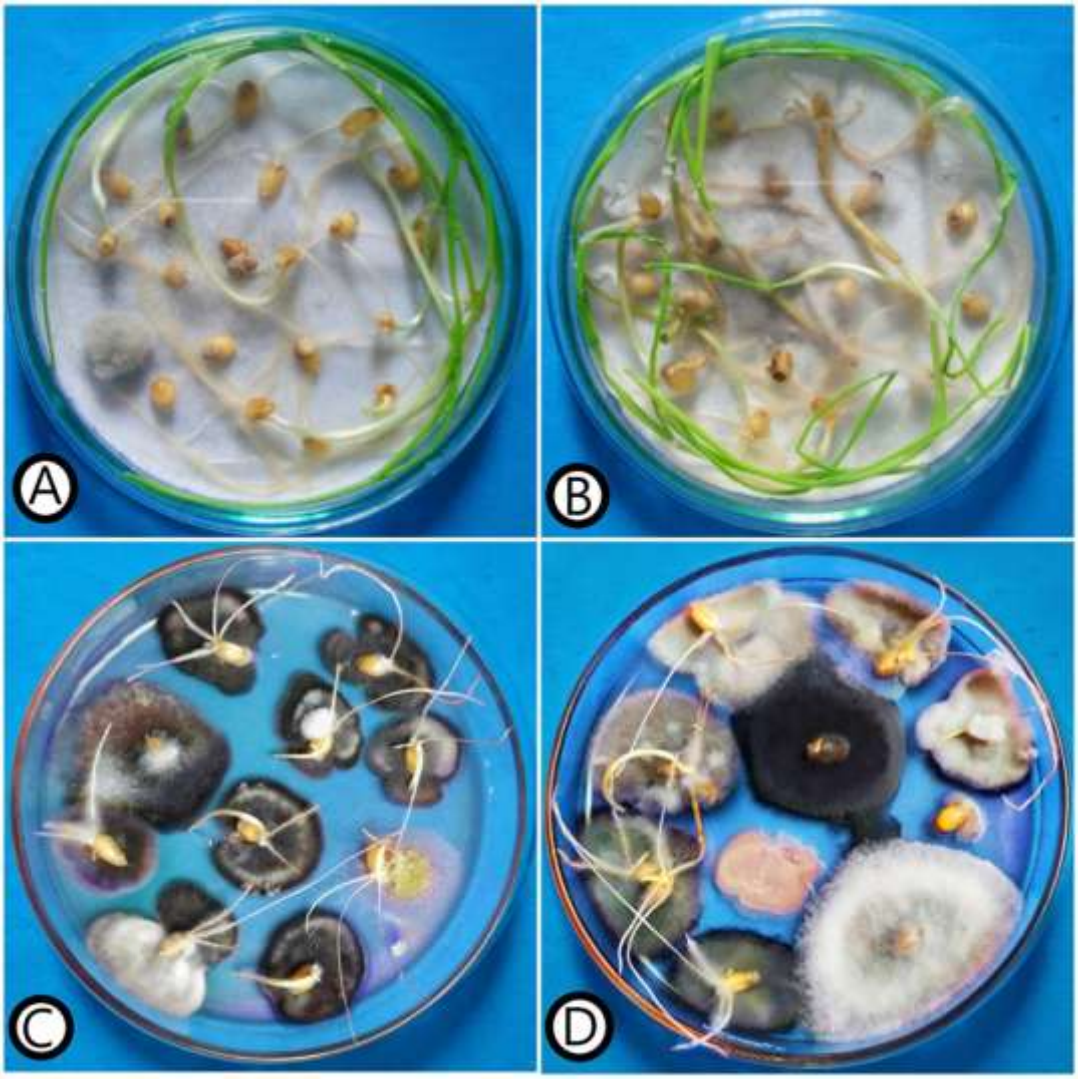

Fig. 7. A and B. Fungi isolation on blotter method; C and D. Fungi isolation on agar plate method from wheat seeds.

The quality of a seed is multiple concept comprising several aspects, such as genetic purity, physical purity, high level of germination and vigour and free from disease, insects etc. Under favourable conditions, the quality of seed is influenced by the kind of crop management practices, storage conditions apart from physical, biochemical and physiological factors of seeds (Doijoide 1988). Among these factors, storage condition plays a major role as it is associated with the attack of storage fungi under variable influences of temperature, relative humidity and seed moisture. The

prevalence of fluctuating storage conditions not only cause significant deterioration in seeds, but also make them useless for sowing in subsequent seasons. Varshney (1990) reported that seed abnormality due to the influence of seed-borne fungi is very common and often accounts for a large percentage of crop losses. Owolade *et al.* (2001) reported that the type and severity of seed abnormalities may be dependent on the type and pathogenic potential of the associated fungi as well as the prevailing weather conditions. Although the range of the fungi associated with wheat seed abnormalities have been reported (Prescott *et al.*1986, Wiese *et al.* 1987, Varshney 1990), there is need to identify and determine the effects of the fungi associated with the abnormalities of wheat seeds.

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