Health and Quality of Wheat Seed Samples Collected from Sadar Upazilla of Thakurgaon District and Control of Seed-Borne Fungi

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Authors’ contributions

This work was carried out in collaboration among all authors. Author MAJ designed the study, wrote the protocol, conducted field experiment, performed the statistical analysis and wrote the first draft of the manuscript. Authors NM, MMR and MGH managed the literature searches, conducted field experiment, collected data, and assisted in data analysis and report writing. All authors read and approved the final manuscript.

ABSTRACT

Wheat seed samples were collected from ten unions of sadar upazilla of Thakurgaon district in wheat growing season of 2011. Seeds were tested by blotter method at Seed Pathology Center (SPC), Bangladesh Agricultural University, Mymensingh during the months April to November 2011 for recording and identifying the seed-borne fungi associated with wheat seeds. The health status of 20 seed samples were determined whereas five genera fungi were identified from a total of six fungus. The fungi were Bipolaris sorokiniana, Alternaria tenuis, Fusarium spp, Penicillium sp, Aspergillus flavus and Aspergillus niger. Prevalence of the total as well as the individual seed-borne fungal infections that were recorded varied significantly with respect to wheat varieties and

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1. INTRODUCTION

Wheat (Triticum aestivum L.) is the most important grain crop providing nearly 20% of the total world food requirement. It is considered as the second most staple food crop next to rice (Oryza sativa L.) in Bangladesh. Besides human nutrition, wheat is also used as animal feed. Wheat straw used as fuel and straw shade have gained much popularity among the poor farmers of Bangladesh. The annual production of wheat in 2010-2011 was 93.4 Million Metric tons obtained from 41.5 Million/ha of land with an average yield of 2.25 metric tons/ha food grain in the world [1]. Next to rice it is second major cereal crop in Bangladesh. Average yield of wheat is low compared to other wheat growing countries of the world. The average yield of wheat is 7.50, 6.20 and 4.80 tons/ha in Holland, UK and France respectively; it is only 2.10 tons/ha in Bangladesh [2].

Poor seed health and quality are also an important factors for the low yield of wheat. The crop suffers from at least 17 different diseases in the country. Twelve of them are caused by fungi. Among the fungal diseases five are considered to be economically important because of their damaging nature and wide occurrence throughout the wheat growing areas of the country [3]. The intimate association of host and parasite in seed provides maximum opportunity for the earliest possible infection of the emerged seedlings. The infected seeds fail to germinate or seedlings and plants developed in the field from infected seeds may escape early infection but may often be infected at the latter stage of growth. Besides, pathogens can spread over a long distance and uninfected field may be infected by the seeds in which different pathogens are present [4]. Disease plays an important role in lowering wheat yields. A considerable number of seed borne fungal pathogens belonging to the genera Bipolaris, Alternaria, Aspergillus, Curvuluria, and Fusarium have been detected in wheat seeds by many researchers [5]. Field fungi associated with seeds may cause deterioration of seed quality affecting viability and reduction in germination.

So far an appreciable amount of work has been done on the control of seed-borne pathogens of wheat by fungicidal seed treatment [6]. Earlier chemical control of plant diseases was quite popular for reducing crop losses. But now-a-days use of chemical for management of crop diseases is being discouraged due to health hazard and environmental pollution and the obvious development of tolerant pathogen; in addition the fungicides are very expensive, alternative means of seed treatment i.e. use of environmental friendly botanical pesticides or plant extract have drawn attention of Plant Pathologists all over the world. Use of plant extracts for seed treatment may open a new era in controlling seed-borne fungal pathogens. In Bangladesh, very few attempts have been made to evaluate the effect of plant extracts against wheat diseases. Efforts for controlling wheat diseases through seed treatment have been made by Forster and Schaad [7].

In view of the above facts, the present study was undertaken to achieve the following objectives to:

1. detect the fungi associated with wheat seeds collected from ten unions of sadar upazilla of Thakurgaon district;
2. record the germination percentage and vigor index of wheat seeds collected from sadar upazilla of Thakurgaon district and
3. know the efficacy of neem leaf extract, hot water and Provax to control seed-borne fungi associated with wheat seeds.

2. MATERIALS AND METHODS

The experiment was conducted at Mycology Lab., Seed Pathology Center (SPC) and M.S. laboratory, Department of Plant pathology, Bangladesh Agricultural University (BAU), Mymensingh. The experiment was conducted during the period from April, 2011 to November, 2011. The materials were used two wheat varieties were used viz. (i) Hazar-8 and (ii) Satabdi.

The collection of seed samples were a total of 20 (10 samples for Hazar-8 and 10 for Satabdi) seed samples of wheat (Triticum aestivum L.) were collected from 10 unions of sadar upazilla of Thakurgaon district. From each union four farmers were selected randomly for collection of seed samples. Then about 0.5 kg of wheat seeds was collected from each farmer house and mixed up thoroughly which is representing a sample for each union. Similarly this process was followed for sample collection in other unions for both varieties. The seed samples were enclosed in polythene bags with proper labeling and were brought directly to SPC laboratory and stored in the refrigerator at 4°C till the seeds were used for the subsequent studies.

The experiment was conducted in a Completely Randomized Design (CRD) with 4 replications. All the seed samples were assayed for the presence of fungal pathogens by the blotto method following the International Rules for Seed Testing Association with slight modification [8]. Seed health status was carried out by blotte method to detect the seed-borne fungi associated with the wheat seed samples. In this method, one hundred seeds were randomly taken from each sample. The seeds were placed on water soaked three layered Whatman No.1 filter paper in plastic petridish. In each petridish, 25 seeds were plated at equal distance. All these petridishes were incubated at 20±2°C under 12 hours alternate cycle of Near Ultra Violet (NUV) light and darkness. After 7-10 days of incubation, petriplates containing incubated seeds were observed under stereomicroscope for detecting seed-borne fungi in wheat seeds surface under stereomicroscope at 25 times magnification. Where identification was difficult or doubtful under the stereomicroscope, temporary slide was prepared and examined under the compound microscope and identified with the help of keys. Number of germinated seeds was recorded along with the seed-borne fungi after 7-10 days of incubation. The results were expressed in percentage.

Preparation of Potato Dextrose Agar (PDA): 200g peeled and sliced potato was boiled in 500 ml water in a bowl for about half an hour. Then the extract of the potato was filtered through cheese cloth. The other two ingredients 20 g dextrose and 20 g agar were added in the extract and the volume was made up to 1L mark. Then the prepared standard PDA was poured in 1000ml conical flask and sterilized (121°C, 121 psi for 45 min.) in an Autoclave. The fungi associated with wheat seeds were inoculated onto PDA plate after taking from infected seeds and were grown as a colony and the photographs were taken.

Assessment of germination percentage: Germination test of the collected seed samples were done in sand using tray method. Plastic trays (18"×9") were used for this purpose. Sand used in this method was collected from the bank of Brahmaputra River besides BAU campus, Mymensingh. Fifty seeds were sown in sand in each plastic tray in five lines (10 seeds/ line) with four replications. For each sample, 200 seeds were tested for germination. Germination was recorded twice at 4 and 8 days after sowing. Total germinated seeds, normal seedlings, abnormal seedlings; non germinated seed were counted and expressed in percentage. Then data on number of normal seedlings, abnormal seedlings and non germinated seeds were recorded according to ISTA rules (2001) [9].

The normal seedlings were categorized by the following criteria as described.

1) Intact seedlings with all essential structures well developed, complete in portion and healthy
2) Seedlings with slight defects are (a) primary root defective but with limited damage or slight growth reduction (b) primary root defective but with sufficient well developed
3) Seedling with secondary infection that would have fallen into category 2 or 1 but for infection by fungi or bacteria from sources other than parent seed.

Abnormal seedlings were categorized using following criteria as described.
1) Seminal root missing / stunted or broken and decayed due to primary infection
2) Coleoptiles missing / split and deformed or bent over
3) Shot system (the mesocotyle if developed) broken / decayed
4) Leaf missing / extending less than halfway up the coleoptiles, shredded or deformed.
5) Seedling as a whole deformed spindly, discolored or decayed as a result of primary infection

The number of seeds that produced normal seedlings was counted and the percentage calculated over the number of seeds placed for the test.

Vigor test: Ten normal seedlings from each tray were taken and their individual shoot and root length were measured. After 8 days length of shoot was measured from the base of the stem up to 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. Shoot and roots were separated from the seedlings. Seedling vigor was determined by the following formula given by Baki and Anderso [10]:

\[
\text{Vigor Index} = (\text{mean of root length} + \text{mean of shoot length}) \times \% \text{ seed germination}
\]

Treatments: Based on the results of analysis of seed health test, one seed sample from the union of each variety of wheat (Triticum aestivum L.) having highest infection by the major seed-borne fungi were selected for this study. The seed samples were treated with neem leaf extract and one physical and one chemical method were used to control the seed-borne fungal infection of wheat seeds. The treatments were: T1 = Control; T2 = Neem leaves extract @ 1:2 (extract: water); T3 = Hot water (56°C for 15 minutes) and T4 = Provax-200® (@ 0.4% of seed weight).

Preparation of neem extract: The neem leaves were collected from the campus of Bangladesh Agricultural University, Mymensingh for this study. The collected plant leaves were chopped after cleaning under running tap water. The extract was prepared by crushing the plant parts in a blender with distilled water at 1:2 (100 g crushed neem leaf in 200 ml water) dilution. The extracts were filtered through cheese cloth. The extracts thus obtained were kept in a refrigerator at 4±1°C until use (Fig. 1).

Seed treatment with neem leaf extract: Seed samples were treated following dipping method. The seeds were dipped into previously prepared recommended dose of 1:2 dilutions of neem leaf extract for 30 minutes. After proper covering of the seed coat with the extract, then the neem leaf extract was drained out from the petriplates. The healths of treated seeds were tested following the standard blotter method with slight modification (ISTA, 2001). After 7-10 days incubation the fungi and germination percentage of seeds were observed and recorded (Figs. 1&2).

Seed treatment with hot water: For hot water treatment of wheat seeds, hot water seed treating device developed at IPM Lab, BAU, Mymensingh was used (Fig. 3).

Hot water treatment of seeds was conducted by following steps:

a. About two liters water was put in the hot water seed treating device.

b. Thermostat valve was adjusted 56°C and switched on the power.

c. Water stirred with glass rod to heat the water uniformly.

d. Sufficient seeds of respective crops in bag were dipped in hot water when the temperature reached at desired level.

e. The bag was stirred so that hot water comes in contact with each seed.

f. After 15 minutes the bag was taken out and seeds were shade dried (Fig. 4).

g. Then the seeds were ready for testing. The health of treated seeds was tested by blotter method and after 7-10 days incubation the fungi and germination percentage of seeds were observed and recorded.

Seed treatment with Provax: One seed treating chemical Provax 200 WP (Hossain Enterprise C.C. Ltd.) was tested @ 0.4% as per direction of the manufacturer against the seed-borne fungal pathogens. Required amount of Provax and seeds from each sample were taken in a 250 ml Erlenmeyer flask and were shaken mechanically for 10 minutes for proper coating of fungicides (Figs. 5 & 6). To check the efficacy of Provax, treated seeds were tested using the Standard Blotter Method with slight modification (ISTA, 1996) [11].

Statistical analysis: The collected data were analyzed by analysis of variance. The mean
3. RESULTS

Altogether six species of fungi were recorded from 20 wheat seed samples collected from ten unions viz., Begunbari, Jagonathpur, Gorea, Salander, Mohamedpur, Rahimunpur, Akcha, Akhanogor, Ruhia of sadar upazilla of Thakurgaon district (Table 1). These were Bipolaris sorokiniana (Figs. 4 & 5), Alternaria tenuis (Figs. 6 & 7), Fusarium spp. (Fig.8&9), Aspergillus flavus (Figs.10 & 11), Aspergillus niger (Figs. 12 & 13), and Penicillum sp.(Figs.14 & 15). Among these fungi, the most prevalent fungi were recorded as Alternaria tenuis (2 to 17%), Aspergillus flavus (5 to 13%), Aspergillus niger (0 to 15%), Bipolaris sorokiniana (15 to 24%), Fusarium moniliformae (0 to 8%), Fusarium oxysporum (0 to 10%) and Penicillum sp. (3 to 12%) in case of Hazar-8 as shown in Table 1.

In case of variety Satabdi, the most prevalent fungi were recorded as Alternaria tenuis (4 to 14%), Aspergillus flavus (4 to 21%), Aspergillus niger (4 to 10%), Bipolaris sorokiniana (10 to 25%), Fusarium moniliformae (0 to 9%), Fusarium oxysporum (0 to 13%) and Penicillum sp. (2 to 12%) as shown in Table 2.

Prevalence of fungi varied significantly among the samples collected from different unions. In case of Hazar-8, the highest (17%) Alternaria tenuis was recorded in seed samples of Gorea union but the lowest (2%) was recorded in seed samples of Salander union (Table 1). The highest (14%) in Gorea union but the lowest (4%) was recorded in Salander union for the variety of Satabdi (Table 2).

After 7 days of incubation the germination percentage ranged from 28 to 90% was recorded in case of variety Hazar-8 and 22 to 95% in Satabdi (Tables 1 & 2). The seed samples obtained from Salander union showed the highest germination percentage for both variety Hazar-8 (90%) and satabdi (95%). The lowest (28%) germination was recorded for Jagonathpur union for variety Hazar-8 and Gorea union (22%) in Satabdi. Finally, seed samples collected from other unions showed moderate and satisfactory germination in case of both varieties Hazar-8 and Satabdi.

The germination percentage of 20 wheat seed samples varied from 58 to 86% in case of variety Hazar-8 (Table 3) and 60 to 90% for the variety Satabdi (Table 4). The highest (86%) germination was recorded in seed samples of Rahimunpur union and the lowest (58%) germination was recorded in seed samples collected from Ruhia union in case of Hazar-8 as shown in (Table 3). In case of Satabdi, the highest (90%) germination was found in seed samples collected from Salander union and the lowest (60%) germination was recorded in seed samples collected from Begunbari and Akcha unions (Table 4).

The range of normal seedlings raised from the collected seed samples was from 48 to 80% for Hazar-8 and 52 to 74% in case of variety Satabdi. The result showed that the highest (80%) of normal seedlings were recorded in seed samples of Rahimunpur union and the lowest (48%) normal seedlings were obtained from the seed samples of Ruhia union for Hazar-8 (Table 3). The range of abnormal seedlings raised from the collected seed samples ranged from 6 to 14% for Hazar-8 and 4 to 18% in case of Satabdi. The highest (14%) of abnormal seedlings were recorded in seed samples of Salander union and lowest (6%) abnormal seedlings were recorded in seed samples of Begunbari, Rahimunpur and Akcha unions in case of Hazar-8 (Table 3). In case of Satabdi, the highest (18%) of abnormal seedlings were recorded in seed samples of Salander union and lowest (4%) abnormal seedlings were recorded in seed samples were collected from Begunbari union (Table 4).

Root length of the collected seed samples varied from 17.5 to 21.3 cm for Hazar-8 and 15.9 to 20.2 cm in case of Satabdi. The highest (21.3 cm) root length of seedlings was measured in seed samples collected from Begunbari union and lowest (17.5 cm) root length was measured in case of seed samples collected from Salander union for Hazar-8 (Table 3). In case of Satabdi, the highest (20.2 cm) root length was measured in seed samples collected from Begunbari union and the lowest (15.9cm) root length was measured in seed samples collected from Salander union (Table 4).

Shoot length of the seedlings raised from the collected seed samples ranged from 9.73 to 13.2 cm in case of Hazar-8 and 9.9 to 12.43 cm in case of variety Satabdi. The highest (13.2 cm) shoot length of seedlings was measured in seed samples of Akcha union and lowest (9.73 cm)
shoot length of seedlings was measured of seed samples collected from Jagonathpur union in Hazar-8 (Table 3). In case of Satabdi, the highest (12.43 cm) shoot length of seedlings was measured in seed samples of Gorea union and lowest (9.9 cm) shoot length of seedlings was measured in seed samples of Akcha union (Table 4).

Vigor index of the seedlings germinated from seed samples collected from Sadar upazilla of Thakurgaon district varied from 1809 to 2785.54 for Hazar-8 and 1680 to 2574.80 in case of Satabdi. The highest (2785.54) vigor index of seedlings was calculated in case of seed samples collected from Rahimunpur union and the lowest (1809.2) vigor index of seedlings was calculated in case of seed samples collected from Ruhia union for Hazar-8 (Table 3). In case of Satabdi, the highest (2574.80) vigor index of seedlings was calculated in seed samples of Akhanogor union and lowest (1680) vigor index of seedlings was calculated in seed samples of Akcha union (Table 4).

Fig. 1. a - Pure culture of Bipolaris sorokiniana, b - Conidiophores and conidia of Bipolaris sorokiniana (x40), c - Pure culture of Alternaria tenuis, d - Conidia of Alternaria tenuis (x40), e - Pure culture of Fusarium spp., f - Conidia of Fusarium spp. (x40), g - Pure culture of Aspergillus flavus, h - Conidiophore and conidia of Aspergillus flavus (x40), i - Pure culture of Aspergillus niger, j - Conidiophore and conidia of Aspergillus niger (x40), k - Pure culture of Penicillium sp., l - Sterigmata, phialodes, conidiophores and conidia of Penicillium sp. (x40)
Table 1. Seed-borne infection of fungi recorded on wheat seeds collected from ten unions of sadar upazilla of Thakurgaon district (Var. Hazar-8)

| Wheat seed sample | % Germination | % Seed-borne infection | Alternaria tenuis | Aspergillus flavus | Aspergillus niger | Bipolaris sorokiniana | Fusarium moniliforme | Fusarium oxysporum | Penicillium sp. |
|-------------------|---------------|------------------------|-------------------|-------------------|-----------------|----------------------|-------------------|------------------|----------------|
| S1                | 31g           |                        | 13.00b            | 7.00e             | 7.00e           | 15.00d               | 0.00d             | 5.00c            | 7.00c          |
| S2                | 28.0h         |                        | 12.00c            | 10.00c            | 8.00d           | 19.00c               | 2.00c             | 8.00b            | 3.00g          |
| S3                | 34.00f        |                        | 8.00d             | 13.00a            | 9.00c           | 18.00c               | 8.00a             | 2.00d            | 4.00f          |
| S4                | 88.00b        |                        | 17.00a            | 7.00e             | 7.00e           | 18.00c               | 2.00c             | 5.00c            | 3.00g          |
| S5                | 90.00a        |                        | 2.00g             | 5.00g             | 0.00g           | 21.00b               | 0.00d             | 5.00c            | 5.00e          |
| S6                | 50.00e        |                        | 12.00c            | 8.00d             | 11.00b          | 16.00d               | 0.00d             | 0.00e            | 3.00g          |
| S7                | 31.00g        |                        | 6.00f             | 11.00b            | 6.00f           | 21.00b               | 0.00d             | 8.00b            | 11.00b         |
| S8                | 85.00c        |                        | 6.00f             | 7.00e             | 7.00e           | 24.00a               | 0.00d             | 10.00a           | 5.00e          |
| S9                | 83.00cd       |                        | 6.00f             | 6.00f             | 8.00d           | 18.00c               | 0.00d             | 0.00e            | 12.00a         |
| S10               | 83.00d        |                        | 7.00e             | 10.00c            | 15.00a          | 18.00c               | 3.00b             | 0.00e            | 6.00d          |
| LSD0.05           | 1.995         |                        | 0.5448            | 0.4899            | 0.6958          | 1.635                | 0.3947            | 0.5130           | 0.5370         |
| CV%               | 2.28          |                        | 4.22              | 4.02              | 6.15            | 6.00                 | 18.16             | 8.22             | 6.28           |

**Significance at 1% level of probability:**

* Similar letter(s) in a column are statistically identical

*S1=Begunbari, S2=Jagonathpur, S3=Nargun, S4=Gorea, S5=Mohamedpur, S6=Rahimunpur, S7=Akcha, S8=Akhanogor, S9=Ruhia*

Table 2. Seed-borne infection of fungi recorded on wheat seeds collected from ten unions of sadar upazilla of Thakurgaon district (Var. Satabdi)

| Wheat seed sample | % Germination | Alternaria tenuis | Aspergillus flavus | Aspergillus niger | Bipolaris sorokiniana | Fusarium moniliforme | Fusarium oxysporum | Penicillium sp. |
|-------------------|---------------|-------------------|-------------------|-----------------|----------------------|-------------------|-------------------|----------------|
| S1                | 58.00e        | 7.00e             | 10.00e            | 8.00c           | 13.00d               | 5.00b             | 3.00d             | 5.00f          |
| S2                | 56.00e        | 5.00g             | 4.00h             | 8.00c           | 10.00e               | 4.00c             | 10.00c            | 6.00e          |
| S3                | 33.00f        | 9.00c             | 15.00b            | 5.00e           | 25.00a               | 9.00a             | 2.00e             | 2.00h          |
| S4                | 22.00g        | 14.00a            | 21.00a            | 10.00a          | 20.00b               | 0.00e             | 11.00b            | 12.00a         |
| S5                | 95.00a        | 4.00h             | 9.00f             | 6.00d           | 20.00b               | 0.00e             | 13.00a            | 2.00h          |
| S6                | 87.00b        | 5.00g             | 14.00c            | 6.00d           | 18.00c               | 4.00c             | 0.00f             | 7.00d          |
| S7                | 82.00c        | 7.00e             | 6.00g             | 8.00c           | 20.00b               | 3.00d             | 0.00f             | 8.00c          |
| S8                | 74.00d        | 8.00d             | 9.00f             | 4.00f           | 20.00b               | 4.00c             | 0.00f             | 7.00d          |
| Wheat seed sample | % Germination | Alternaria tenuis | Aspergillus flavus | Aspergillus niger | Bipolaris sorokiniana | Fusarium moniliforme | Fusarium oxysporum | Penicillium sp. |
|-------------------|---------------|-------------------|-------------------|------------------|-----------------------|---------------------|-----------------|----------------|
| S1                | 88.00b        | 6.00f             | 10.00e            | 8.00c            | 17.00c                | 3.00d               | 0.00f           | 9.00b          |
| S10               | 73.00d        | 10.00b            | 11.00d            | 9.00b            | 20.00b                | 5.00b               | 0.00f           | 3.00g          |
| LSD0.05           | 2.583         | 0.517             | 0.6913            | 0.5822           | 1.202                 | 0.3893              | 0.4790          | 0.6308         |
| Level of significance ** | ** ** ** ** ** ** ** ** ** | ** ** ** ** ** ** ** ** ** ** | ** ** ** ** ** ** ** ** ** ** | ** ** ** ** ** ** ** ** ** ** | ** ** ** ** ** ** ** ** ** ** | ** ** ** ** ** ** ** ** ** ** | ** ** ** ** ** ** ** ** ** ** | ** ** ** ** ** ** ** ** ** ** |
| CV%               | 2.67          | 4.75              | 4.37              | 5.57             | 4.53                  | 7.24                | 8.47            | 7.12           |

** Significance at 1% level of probability
* Similar letter(s) in a column are statistically identical

Table 3. Per cent germination of wheat seed and vigor index of seedlings raised from wheat seeds collected from ten unions of sadar upazilla of Thakurgaon district (Var. Hazar-8)

| Wheat seed sample | % Germination | % Normal seedling | % Abnormal seedling | % Non germinated seed | Root length (cm) | Shoot length (cm) | Vigor index |
|-------------------|---------------|-------------------|--------------------|----------------------|------------------|-------------------|-------------|
| S1                | 78.00b        | 72.00b            | 6.00e              | 22.00f               | 21.30a           | 12.61a            | 2644.98b    |
| S2                | 64.00e        | 56.00e            | 8.00d              | 36.00c               | 19.04bc          | 9.730d            | 1864.32g    |
| S3                | 74.00c        | 64.00cd           | 10.00c             | 26.00e               | 19.10bc          | 12.65a            | 2342.10de   |
| S4                | 78.00b        | 66.00c            | 12.00b             | 22.00f               | 19.50b           | 11.63b            | 2428.14cd   |
| S5                | 80.00b        | 66.00c            | 14.00a             | 20.00g               | 19.17bc          | 11.71bc           | 2293.60e    |
| S6                | 84.00a        | 72.00b            | 12.00b             | 16.00h               | 19.17bc          | 10.49cd           | 2491.44c    |
| S7                | 86.00a        | 80.00a            | 6.00e              | 14.00i               | 19.35b           | 13.04a            | 2785.54a    |
| S8                | 60.00f        | 54.00e            | 6.00e              | 40.00b               | 18.48bc          | 13.20a            | 1900.80g    |
| S9                | 70.00d        | 62.00d            | 8.00d              | 30.00d               | 19.25bc          | 10.65c            | 2093.00f    |
| S10               | 58.00f        | 48.00f            | 10.00c             | 42.00a               | 18.67bc          | 12.52a            | 1809.02g    |
| LSD0.05           | 2.683         | 2.988             | 0.8000             | 1.450                | 1.563            | 0.8729            | 96.65       |
| Level of sig **   | ** ** **     | ** ** **          | ** ** **           | ** ** **             | ** ** **         | ** ** **          | ** ** **    |
| CV%               | 2.53          | 3.22              | 5.99               | 3.73                 | 5.63             | 5.11              | 2.94        |

** Significance at 1% level of probability
* Similar letter(s) in a column are statistically identical

S1=Begunbari, S2=Jagonathpur, S3=Nargun, S4=Gorea, S5=Salander, S6=Mohamedpur, S7=Rahimunpur, S8=Akcha, S9=Akhanogor, S10=Ruhia
Table 4. Per cent germination of wheat seed and vigor index of seedlings raised from wheat seeds collected from ten unions of sadar upazilla of Thakurgaon district (Var. Satabdi)

| Wheat seed sample | % Germination | % Normal seedling | % Abnormal seedling | % Non germinated seed | Root length (cm) | Shoot length (cm) | Vigor index |
|-------------------|---------------|-------------------|---------------------|-----------------------|------------------|------------------|-------------|
| S1                | 60.00e        | 56.00c            | 4.00f               | 40.00a                | 20.20a           | 11.65b           | 1911.00ef   |
| S2                | 62.00de       | 54.00cd           | 8.00d               | 38.00ab               | 18.70bc          | 11.35b           | 1836.00f    |
| S3                | 82.00b        | 72.00a            | 10.00c              | 18.00d                | 19.00abc         | 11.30b           | 2488.79a    |
| S4                | 70.00c        | 56.00c            | 14.00b              | 30.00c                | 19.30abc         | 12.43a           | 2224.60c    |
| S5                | 90.00a        | 72.00a            | 18.00a              | 10.00e                | 15.90e           | 10.25cd          | 2353.50b    |
| S6                | 70.00c        | 62.00b            | 8.00d               | 30.00c                | 19.50ab          | 11.35b           | 2096.50d    |
| S7                | 68.00c        | 62.00b            | 6.00e               | 32.00c                | 19.05abc         | 10.75c           | 2026.50de   |
| S8                | 60.00e        | 52.00d            | 8.00d               | 40.00a                | 18.10cd          | 9.900d           | 1680.00g    |
| S9                | 82.00b        | 74.00a            | 8.00d               | 18.00d                | 19.05abc         | 11.30b           | 2574.80a    |
| S10               | 64.00d        | 54.00cd           | 10.00c              | 36.00b                | 17.00de          | 11.70b           | 1836.80f    |
| LSD0.05           | 2.923         | 3.234             | 0.6521              | 2.108                 | 1.295            | 0.5088           | 127.5       |
| Level of significance | **         | **                | **                  | **                    | **               | **               | **          |
| CV%               | 2.85          | 3.63              | 4.78                | 4.78                  | 3.15             | 4.18             |             |

** Significance at 1% level of probability
* Similar letter(s) in a column are statistically identical

S1=Begunbari, S2=Jagonathpur, S3=Nargun, S4=Gorea, S5=Salander, S6=Mohamedpur, S7=Rahimunpur, S8=Akcha, S9=Akhanogor, S10=Ruhia

Table 5. Effect of neem leaf extract, hot water and provax in control of seed-borne fungi of wheat seeds (Var. Hazar-8)

| Treatment | % Germination | Alternaria tenuis | Aspergillus flavus | Aspergillus niger | Bipolaris sorokinonia | Fusarium moniliformae | Fusarium oxysporum | Penicillium sp. |
|-----------|---------------|-------------------|-------------------|------------------|-----------------------|----------------------|-------------------|----------------|
| T1        | 76.00b        | 11.00a            | 10.00a            | 9                | 21.00a                | 2                    | 0                 | 8              |
| T2        | 79.00b        | 2.00b             | 2.00c             | 0                | 2.00c                 | 0                    | 0                 | 0              |
| T3        | 87.00a        | 2.00b             | 3.00b             | 0                | 5.00b                 | 0                    | 0                 | 0              |
| T4        | 84.00a        | 0.00c             | 0.00d             | 0                | 0.00d                 | 0                    | 0                 | 0              |
| LSD0.05   | 3.317         | 0.4078            | 0.7189            | 0.9063           |                       |                      |                   |                |
| Level of sig | **          | **                | **                | **               | **                    | **                   |                   |                |
| CV%       | 2.58          | 6.81              | 11.98             | 8.09             |                      |                      |                   |                |

** Significance at 1% level of probability
* Similar letter(s) in a column are statistically identical

T1=Control, T2=Neem leaf extract, T3=Hot water, T4=Provax (0.04%)
Table 6. Effect of neem leaf extract, hot water and provax in control of seed-borne fungi of wheat seeds (Var. Satabdi)

| Treatment | % Germination | Alternaria tenuis | Aspergillus flavus | Aspergillus niger | Bipolaris sorokiniana | Fusarium moniliformae | Fusarium oxysporum | Penicillium sp. |
|-----------|---------------|-------------------|-------------------|------------------|-----------------------|-----------------------|-------------------|--------------|
| T₁        | 43.00c        | 10                | 14.00a            | 11               | 21.00a                | 2                     | 0                 | 9            |
| T₂        | 83.00a        | 0                 | 3.00c             | 0                | 3.00c                 | 0                     | 0                 | 0            |
| T₃        | 83.00a        | 0                 | 5.00b             | 0                | 6.00b                 | 0                     | 0                 | 0            |
| T₄        | 76.00b        | 0                 | 0.00d             | 0                | 0.00d                 | 0                     | 0                 | 0            |
| LSD0.05   | 6.017         | 0.8281            |                   |                  | 0.9873                |                       |                   |              |
| Level of sig. | **      | **                |                   |                  | **                   |                       |                   |              |
| CV%       | 5.28          | 9.41              |                   |                  | 8.23                 |                       |                   |              |

** Significance at 1% level of probability
* Similar letter(s) in a column are statistically identical
T₁=Control, T₂=Neem leaf extract, T₃=Hot water, T₄=Provax (0.04%)
The neem leaf extract at 1:2 dilution was found effective in reducing the seed-borne fungal infection in seeds of many crop species (Fakir and Khan, 1992). Hence, in this study neem leaf extract at 1:2 dilution was selected as one of the approach for treating wheat seeds. Neem leaf extract at 1:2 dilution significantly reduced the different seed-borne fungal infections. The results have been presented in Tables 5 and 6.

It was observed that the treated seeds showed significantly higher percent of germination than untreated seeds. Germination was recorded (79%) in variety Hazar 8 and (83%) in Satabdi when the seeds were treated with neem leaf extract at 1:2 dilution (Tables 5 and 6).

It was found that the treated seeds showed significantly higher germination than untreated seeds and the results were statistically significant. The per cent germination of seed increased 87% in Hazar-8 and 83% in case of satabdi when seeds were treated with hot water at 56°C for 15 min. The prevalence of three fungi Bipolaris sorokiniana (5%), Aspergillus flavus (3%) and Alternaria tenuis (2%) were recorded at minimum level in case of Hazar-8. The presence of Aspergillus niger, Fusarium spp and Penicillium sp were recorded highest in control and these were completely controlled when seeds were treated with hot water at 56°C for 15 min (Table 5). In case of Satabdi, the infection of Aspergillus flavus (14%) and Bipolaris sorokiniana (21%) were recorded in control but when seeds were treated with hot water Aspergillus flavus and Bipolaris sorokiniana was found at minimum level (5%) and (6%), respectively. The prevalence of Alternaria tenuis, Aspergillus niger, Fusarium spp. and Penicillium sp were recorded highest in control but these were completely controlled when seeds were treated with hot water at 56°C for 15 min (Table 6).

Significant effect of seed treatment with Provax was observed on the prevalence of seed-borne fungi associated with seeds (Table 5 and 6). It was observed that the treated seeds resulted in 4% germination in Hazar-8 and 76% germination in case of Satabdi. Significantly higher percentage of germination was recorded in the treated seeds than untreated seeds both in Hazar-8 and in Satabdi (Table 5 and 6).

4. DISCUSSION

The present findings clearly showed that the fungi Bipolaris sorokiniana, Alternaria tenuis, Fusarium spp., Penicillium sp., Aspergillus niger and Aspergillus flavus associated with the wheat seed samples and significantly reduced the percentage of seed germination in both Hazar-8 and Satabdi. This difference might be due to the procedural differences in sample collection, differences in storage condition as well as the quantity and kinds of seed-borne fungal flora associated with them. The yield and yield contributing characters of many crop species largely depends on the percent germination, normal and abnormal seedlings raised from the seeds. The results showed that the highest germination was recorded in seed samples of Rahimunpur union and the lowest germination was recorded in seed samples collected from Ruhia union in case of Hazar-8. In case of Satabdi, the highest germination was found in seed samples collected from Salander union and the lowest germination was recorded in seed samples collected from Begunbari and Akcha unions. The highest normal seedlings were recorded from seed samples of Rahimunpur union and the lowest normal seedlings were obtained from the seed samples of Ruhia union in Hazar-8. For Satabdi, the highest normal seedlings were recorded in seed samples of Akhanogor union and the lowest normal seedlings were obtained from the seed samples of Akcha union. The highest abnormal seedlings were recorded in seed samples of Salander union and lowest abnormal seedlings were recorded in seed samples of Begunbari union. The highest abnormal seedlings were calculated from seed samples of Salander union and lowest abnormal seedlings were recorded in seed samples of Begunbari union. On the other hand, vigor index of wheat seedlings raised from the seed samples collected from ten unions of Thakurgaon district were evaluated. The highest vigor index of seedlings was calculated in case of seed samples collected from Rahimunpur union and the lowest vigor index of seedlings was calculated in case of seed samples collected from Ruhia union for Hazar-8. In case of Satabdi, the highest abnormal seedlings was calculated from seed samples of Salander union and lowest abnormal seedlings were recorded in seed samples of Begunbari union. On the other hand, vigor index of seedlings raised from the seed samples collected from ten unions of Thakurgaon district were evaluated. The highest vigor index of seedlings was calculated in case of seed samples collected from Rahimunpur union and the lowest vigor index of seedlings was calculated in case of seed samples collected from Ruhia union for Hazar-8. In case of Satabdi, the highest vigor index of seedlings was calculated in seed samples of Akhanogor union and lowest vigor index of seedlings was calculated in seed samples of Akcha union. These results suggest that seed samples collected from Rahimunpur union and seed samples from Akhanogor union were superior in both Hazar-8 and Satabdi in terms of health and quality.

Seeds were treated with neem leaf extract, hot water and Provax. The results revealed that
neem leaf extract was used for controlling seed-borne fungi of wheat seeds. The neem leaf extract performed better in reducing seed-borne prevalence of all the major fungi and increasing seed germination. Seed treatments with neem leaf extract results in higher germination in different crops including wheat have been reported by Ahmed and Sultana [12] and Khaleduzzaman [13]. Hot water effectively controlled the seed-borne fungi associated with wheat seeds. Provax performed best in controlling B. sorokiniana, A. tenuis, Penicillium sp, Fusarium spp and Aspergillus spp. In respect of the efficacy, Provax stood first in controlling the major seed-borne fungi associated with wheat seeds which corroborate with the findings of Rahman [14] and Sultana [15]. They observed that the prevalence of seed-borne fungi was controlled and seedling mortality of wheat was reduced.

5. CONCLUSION

A total of 20 wheat seed samples were tested for health and quality analysis. Six species of fungi of five genera were identified from these samples. The identified species were Bipolaris sorokiniana, Alternaria tenuis, Fusarium spp., Penicillium sp., Aspergillus flavus and Aspergillus niger. The prevalence of fungi varied significantly in respect of variety and seed source. The results revealed that the highest fungal infections were recorded in the seed samples collected from Jagonathpur union in case of Hazar-8 and in the seed samples from Gorea union in case of Satabdi. The seed-borne fungal infection in Hazar-8 and Satabdi collected from other unions showed lower percentage as compared to Jagonathpur and Gorea union of sadar upazilla of Thakurgaon district.

Of the fungi encountered, Bipolaris sorokiniana and Fusarium spp. showed the highest and lowest percentage of infection, respectively with respect of both variety and location. Germination of seeds was recorded highest in Rahimunpur union and lowest in Ruhia union for the variety of Hazar-8. In case of Satabdi, the highest germination was found in Salander union and lowest in Begunbari and Akcha union.

Therefore, it may be concluded that wheat seeds produced by the farmers in different parts of Bangladesh carry important seed-borne fungi and treating of seeds with Provax and neem leaf extract were found best in controlling fungi of wheat seeds. However, more comprehensive research on the health status of wheat seeds and seed treatment with physical, chemical and biological means need to be investigated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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