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Prevalence of seed-borne *Fusarium* in local rice cultivars from Barishal district

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ABSTRACT

Seed-borne diseases cause substantial yield loss of rice every year. The present study was conducted to determine the status of seed-borne *Fusarium* spp. infection in farmers' saved rice seeds of nine local cultivars collected from all ten upazila of Barishal district. Two fungal species of *Fusarium,* i.e., *Fusarium fujikuroi,* and *Fusarium equiseti* were identified based on morphological characteristics using the blotter method. The highest infection of *F. fujikuroi* was observed in Babuganj upazila (11.11%), followed by Agailjhara (8.83%). On the other hand, the highest infection of *F. equiseti* was observed in Bakerganj (8.17%), followed by Mehendiganj (8.05%). The lowest infection of *F. fujikuroi* and *F. equiseti* was observed in Muladi, with values of 5.22% and 4.54%, respectively. Among local cultivars, the highest infection of *F. fujikuroi* (7.11%) was observed in Lalpaika followed by Sakkhorkora (6.83%). On the other hand, the highest infection of *F. equiseti* was observed in Dudhmona (9.17%) followed by Kajalshail (8.94%). The lowest infection of *F. fujikuroi* and *F. equiseti* was observed in the Bhushihara cultivar with the same infestion (4.61%). The prevalence of *Fusarium* spp. varied with respect to the location of seed collection as well as cultivars. Highest seed germination was observed in Bhushihara (89%) followed by Swarna (87%) and Joyna (85%). Lowest seed germination was observed in Lalpaika (78%). The highest mortality of the seedlings after germination was observed in Lalpaika (24.25%), followed by Dudhmona (23%) and the lowest mortality of the seedlings was observed in Bhushihara (14.75%). Thus, seed germination rate and post-emergence mortality of seedlings were directly associated with seed-borne infection of *F. fujikuroi* and *F. equiseti.*

Introduction

Rice is one of the most important staple foods for more than half of the world population. It is a widely grown cereal crop in tropical and subtropical regions of the world, including Bangladesh. Average rice yield is incredibly poor in Bangladesh when compared to other rice-growing nations worldwide. The disease is one of the important factors lowering the yield of rice in Bangladesh. Globally yield loss in rice due to pathogens has been estimated to be 15%–30%, which costs about 33 billion USD annually (Oerke *et al.,* 2012). Average domestic

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losses due to diseases over the decade 1989- 90 to 1998-99 was estimated as 3% in Boro, 5.9% in Aus, and 6.0% in Aman with an average for three seasons is 4.9%, contributing to an annual loss of 1.52 million ton per year (Islam and Catling, 2012). In Bangladesh, 32 diseases are known to occur in rice. Among these diseases, 10 are of major importance and 22 are minors (BRRI, 2016). The major seed-borne diseases of rice are brown spot (*Bipolaris oryzae*), bakanae (*Fusarium monilliforme*), blast (*Pyricularia oryzae*), sheath rot (*Sarocladium oryzae*), and bacterial leaf bight (*Xanthomonas oryzae* pv. *oryzae*) are associated with infection of rice seeds and causes reduction of yield (Haque *et al.,* 2007). In recent years, the bakanae disease of rice caused by *Fusarium* spp. has been reported to occur frequently. Several fungal species under the genus *Fusarium* such as *F. fujikuroi*, *F. equiseti*, *F. proliferatum, F. oxysporum, F. sacchari, F. subglutinans* and *F. verticillioides, F. graminearum, F. avenaceum, F. solani* and *F. semitectum* were reported to be associated with rice seeds worldwide (Amatulli *et al.,* 2010; Gopalakrishnan *et al.,* 2010; Sultana *et al.,* 2020). Seed-borne *F. fujikuroi* has been reported as a causal agent of bakanae disease but some other species of *Fusarium* such as *F. proliferatum* and *F. verticillioides* have been isolated from rice seeds and found to be associated with Bakanae disease (Amatulli *et al.,* 2010). Rice seeds from public and private institutions have a significant proportion of seed-borne pathogen infestation, and in recent years, the usage of pathogen-infested seeds has led to an increase in the incidence of seed-borne diseases in different rice-growing regions of Bangladesh (Kabilan *et al.,* 2021). The primary source of seeds for rice production in our nation is farmer-saved seeds. However, due to improper seed health maintenance, farmers' saved seeds are heavily infested with fungi, especially during hot and humid seasons, which degrades the quality and viability of the seeds. The infected seeds can't germinate, and transmit pathogens from seed to seedling and from infected seedling to growing plants in the fields. It could be a major source of inoculums for new crops raised from them. Seed-borne pathogens affect the quality of the seed and cause abnormal seedling (Gebeyaw, 2020). So, determination of the health status of rice seeds is necessary to know the seed-borne infection. It is important to know whether a seed lot is free from seed-borne infection of pathogens or not. Without improving the quality of the seed, improved technology can hardly improve the production potential. Barishal district is one of the major rice-growing areas of Bangladesh. Most of the farmers in this region generally used to cultivate local varieties and they use their own preserved seeds for cultivation. The health status of these seeds of local varieties is still unknown. Considering the circumstances above, the main objective of the experiment was to determine the status of seed-borne pathogenic *Fusarium* spp. in farmers' saved rice seeds from Barishal district.

Materials and Methods

Collection of rice seed sample

Altogether 90 rice seed samples of nine popular local cultivars (Sakkhorkora, Dudhmona, Joyna, Sawarna, Sadamota, Kalomota, Bhushihara, Lalpaika and Kajalshail) were collected in Aman season from the farmers of all 10 upazila (Agailjhara, Babuganj, Barisal-Sadar, Bakerganj, Banaripara, Gouranadi, Hizla, Mehendiganj, Muladi and Uzirpur) of Barishal district. Nine seed samples from each upazila were used for the detection of seed-borne *Fusarium* species. Samples were kept in a brown paper bag, appropriately labeled and preserved in a refrigerator for subsequent use.

Prevalence of seed-borne fusarium species

The collected seed samples were tested using the blotter method to detect seed-borne *Fusarium* spp. following the International Rules for Seed Health Testing (ISTA, 1996). Three layers of blotter papers (Whatman No.1) were soaked in sterilized water and then placed at the bottom of a 9-centimeter diameter plastic Petri dish. Four hundred seeds from each sample were taken randomly and then 25 seeds were placed on wet blotter paper in each plate. The Petri dishes containing the seeds were incubated at 27ºC under an alternating cycle of 12/12 hours NUV (near ultra-violet) light and darkness for seven days. After incubation, seeds were examined under the stereo-microscope (4X) to record the incidence of seed-borne *Fusarium* spp. and identified by observing the fungal colony growth characters such as colony morphology, texture and color on the incubated seeds following the standard keys (Mathur and Kongsdal, 2003). In doubtful cases, for proper identification of *Fusarium* spp., temporary slides were prepared from the fungal colony and observed under compound microscope (40X) and identified with the help of standard keys based on conidia formation process, conidial size, shape, color, number of septation, pattern of conidial arrangement in conidiophore (Booth, 1971; Mathur and Kongsdal, 2003). The results were presented as percent infection for individual *Fusarium* species. The germination percentage of the seeds was recorded. After ten days of incubation, the number of dead seedlings due to infection was counted and expressed as a mortality percentage (ISTA, 1996). The relationship between seed germination failure and total seed-borne infections of *Fusarium* spp. was also assessed.

Design of experiment and data analysis

The experiment was conducted using a completely randomized design (CRD) with three replications. Data resulting from the experiment were arranged in MS Office Excel 2019 software. The data were analyzed using one-way ANOVA and LSD test, $P<0.05$ was considered statistically significant. Statistics-10 software was used for statistical analysis.

Results and Discussion

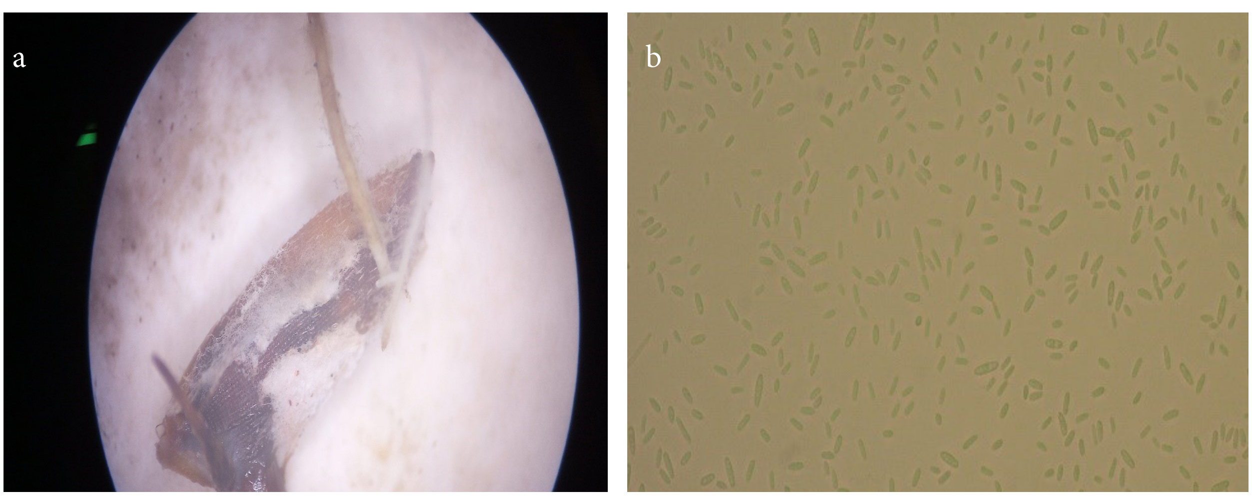
Detection and identification of seed-borne Fusarium species

Seed-borne *Fusarium* species in farmers' rice seeds of nine local cultivars that were collected from all upazila of Barishal district of Bangladesh in Aman season were detected and identified by blotter method.

Two fungal species of *Fusarium* i.e., *Fusarium fujikuroi,* and *F. equiseti* were identified based on the morphological characteristics observed under stereo and compound microscope. Stereomicroscopic and compound microscopic characteristics of *F. fujikuroi* and *F. equiseti* are described in Table 1 and their pictorial observation is illustrated in Fig.1 (a, b, c and d).

Table 1. Stereo and compound microscopic characteristics of the identified *Fusarium fujikuroi* and *F. equiseti*

Observation	Characteristics	Identified as
Stereomicroscopic	Cottony-white with a slightly dark fungal mycelial colony on the seed.	*F. fujikuroi*
Compound-microscopic	Microconidia (7.5-12.3×1.9-3.8µm) were single-celled, oval-shaped with distinct flattened bases. Macroconidia (16.3-30.5×1.9-3.8µm) were delicate, almost straight, 3-5 septate.	
Stereomicroscopic	Bright-white with the floccose fungal mycelial colony on the seed.	*F. equiseti*
Compound-microscopic	Macroconidia (20.5–55.5×2.2–4.8µm) were produced and were characterized as delicate, sickle-shaped, 3-5 septate with distinct foot-shaped cell at the base. Macroconidia were slightly curved at the apex microconidia were absent. Chlamydospore was observed.	



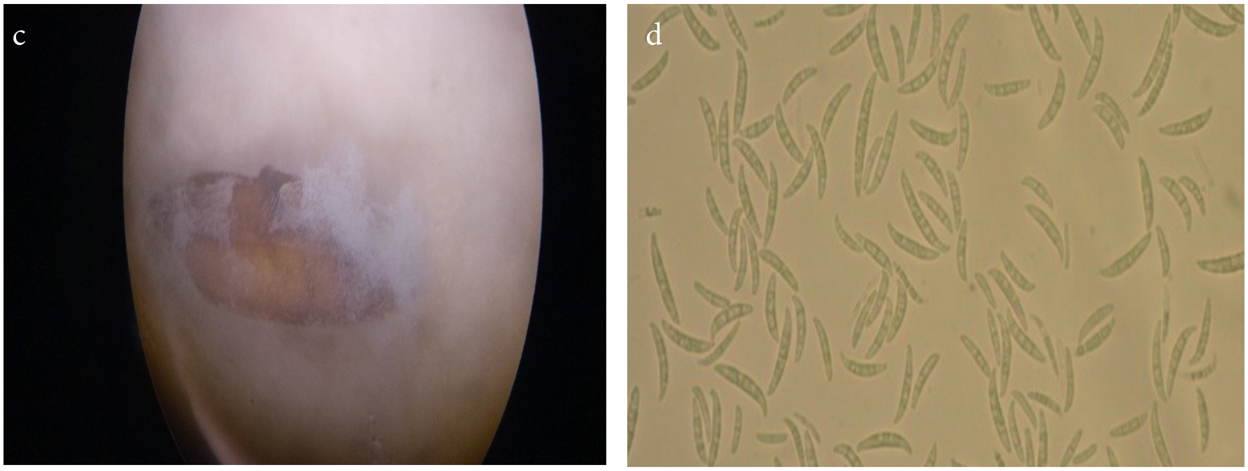


Fig. 1. Stereomicroscopic view (4X) and compound microscopic view (40X) of the identified *F. fujikuroi* (a, b) and *F. equiseti* (c, d).

Prevalence of seed-borne fusarium* spp. *in rice seeds

The prevalence percentages of *F. fujikuroi* and *F. equiseti* in all upazila of Barishal district were significantly different from each other (*P<0.05*). The infection percentages of *F. fujikuroi* ranged from 5.22-11.11%. The highest infection of *F. fujikuroi* was observed in Babuganj upazila (11.11%) followed by Agailjhara (8.83%) and Barisal Sadar (7.88%). The infection percentages of *F. equiseti* ranged from and 8.05%) (Fig. 2).4.54-8.17%.

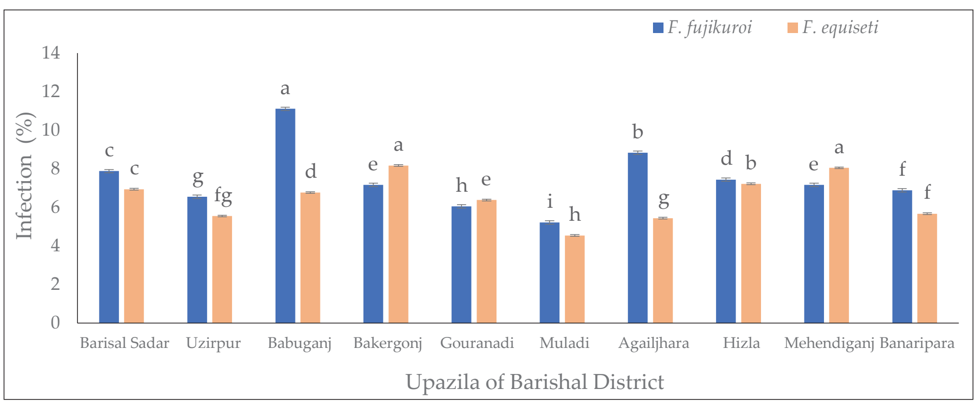


Fig. 2. Association percentage of *F. fujikuroi* and *F. equiseti* in rice seeds of all upazila of Barishal district of Bangladesh. Data were analyzed using LSD tests and means with different letters were significantly different (*P<0.05*).

4.54-8.17%. The highest infection of *F. equiseti* was observed in Bakerganj (8.17%) followed by Mehendiganj (8.05%) and Hizla (7.22%). The lowest infection of *F. fujikuroi* and *F. equiseti* was observed in Muladi with values of 5.22% and 4.54%, respectively. The seed samples from Bakerganj and Mehendiganj showed statistically similar levels of *F. fujikuroi* (7.17%) and *F. equiseti* infection (8.17%

The infection percentages of F. fujikuroi and F. equiseti in farmers' rice seeds of nine local cultivars were significantly different from each other (P<0.05). The infection percentages of F. fujikuroi ranged from 4.61-7.11%. The highest infection of F. fujikuroi was observed in seeds of Lalpaika (7.11%) followed by those of Sakkhorkora (6.83%) and Kalomota (6.11%). The infection percentages of *F. equiseti* ranged from 4.61-9.17%. The highest infection of *F. equiseti* was observed in seeds of Dudhmona (9.17%) followed by those of Kajalshail (8.94%) and Lalpaika (7.77%). The lowest infection (4.61%) of *F. fujikuroi* and *F. equiseti* was observed in seeds of the cultivar Bhushihara. In Swarna and Bhushihara infection of *F. fujikuroi* was statistically nonsignificant with the same values of

4.67% and 4.61%, respectively and infection of *F. fujikuroi* in Joyna and Dudhmona was statistically nonsignificant with values of 5.52% and 5.50%, respectively. Infection of *F. equiseti* was statistically nonsignificant in Lalpaika and Sakkhorkora with values of 7.77% and 7.67%, respectively (Fig. 3).

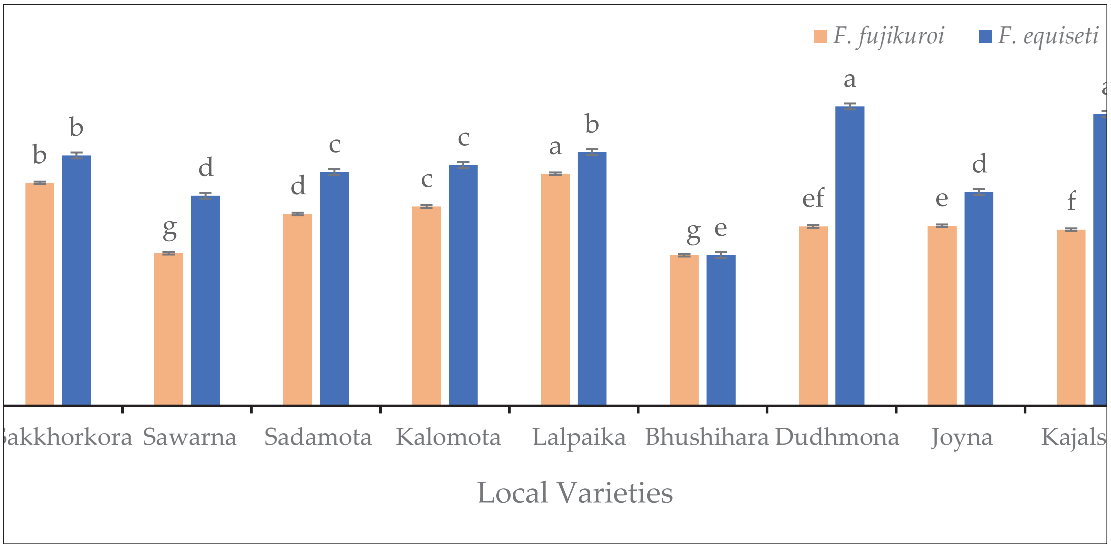


Fig. 3. Percentage of association of *F. fujikuroi* and *F. equiseti* in farmers' rice seeds of nine locally cultivated varieties. Data were analyzed using LSD tests and means with different letters were significantly different (*P<0.05*).

The association of different species of fungi under the genus *Fusarium* with rice seed has been reported by quite a good number of workers. Khan *et al.* (2000) detected seed-borne infection of *F. moniliforme* and *F. semitectum* in rice seed samples from central Punjab of Pakistan. Haque *et el.* (2007) reported that the prevalence of *Fusarium* sp. was 13.5% in the seed samples collected from untrained farmers (seed purpose) and 4.6% in the seed samples of trained farmers (seed purpose) in Babuganj, Barishal. Gopalakrishnan *et al.* (2010) reported that *Fusarium moniliforme* (23%), *F. semitectum* (2.09%) and *F. solani* (3.48%) were associated with the seed samples of rice. *F. moniliforme* was identified in seed samples of hybrid rice varieties of Bangladesh (Ora *et al.,* 2011) and in 15 fine and coarse rice varieties in Pakistan (Habib *et al.,* 2012). Ahmed *et al.* (2013) conducted an experiment to detect the fungal association with the seed samples of three rice varieties (BR6, Joya and Pajam) collected from the Parshuram upazila of Feni district. During the investigation, *F. oxysporum,* and *F. moniliforme* were identified on the seeds of three varieties. The incidence of *F. oxysporum* in the seeds of three rice varieties Joya, BR6 and Pajam was 9.333%, 7.000% and 6.333%, respectively. At the same time, the incidence of *F. moniliforme* in the seeds of three rice varieties Joya, BR6 and Pajam was 6.333%, 6.333% and 4.667%, respectively. According to Sultana *et al.* (2020) four fungal species *Fusarium equiseti, F. fujikuroi, F. oxysporum* and *F. proliferatum* were isolated from the seeds of 20 varieties of rice (BRRIdhan 56 to BRRIdhan 75). Hossain *et al.* (2020) observed that the prevalence of the *F. moniliforme* varied according to locations and seasons in Bangladesh. They also found that infection of *F. moniliforme* was significantly higher in farmers' seeds (8.46%) than in BADC seeds (3.96%).

Germination rate and seedling mortality of farmer rice seeds of nine local cultivars

The germination rate and seedling mortality of each of the local cultivars were significantly different in different areas (*P<0.05*) (Fig. 4 and Fig. 5). The germination rate of the nine local cultivars ranged from 78-89%. The highest seed germination was observed in Bhushihara (89%) followed by Swarna (87%) and Joyna (85%). On the other hand, seed germination of Sakkhorkora and Kajalshail was statistically nonsignificant with the same value (80%). In Sadamota and Joyna, seed germination was not significant with values 84% and 85%, respectively. The lowest seed germination was observed in Lalpaika (78%) (Fig. 4).

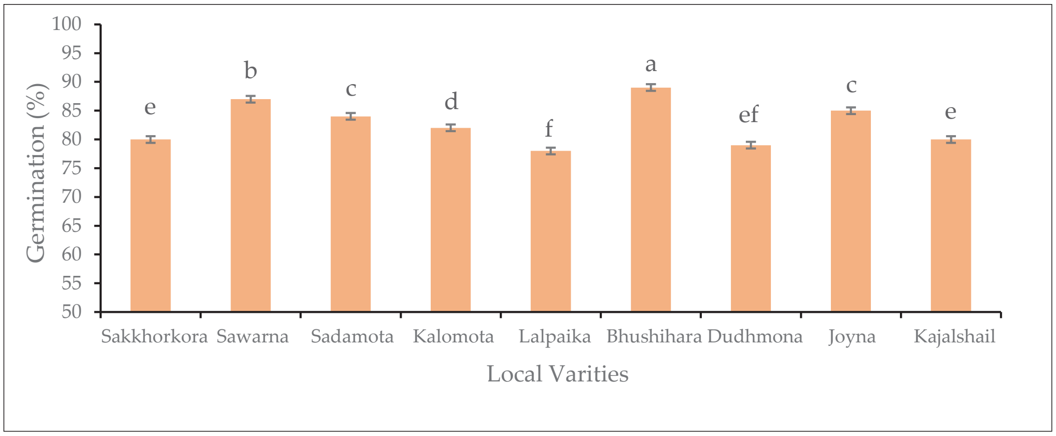


Fig. 4. Germination percentage of farmers' rice seeds of nine locally cultivated varieties. [Bar(s) above which the same letter(s) were not significantly different (*P<0.05*)].

The mortality of rice seedlings ranged from 14.75-24.25%. The highest mortality of the seedlings after germination was observed in Lalpaika (24.25%) followed by Dudhmona (23%), Sakkhorkora (22.25%) and Kajalshail (21%). On the other hand, the lowest mortality of the seedlings (14.75%) was observed in Bhushihara (Fig. 5).

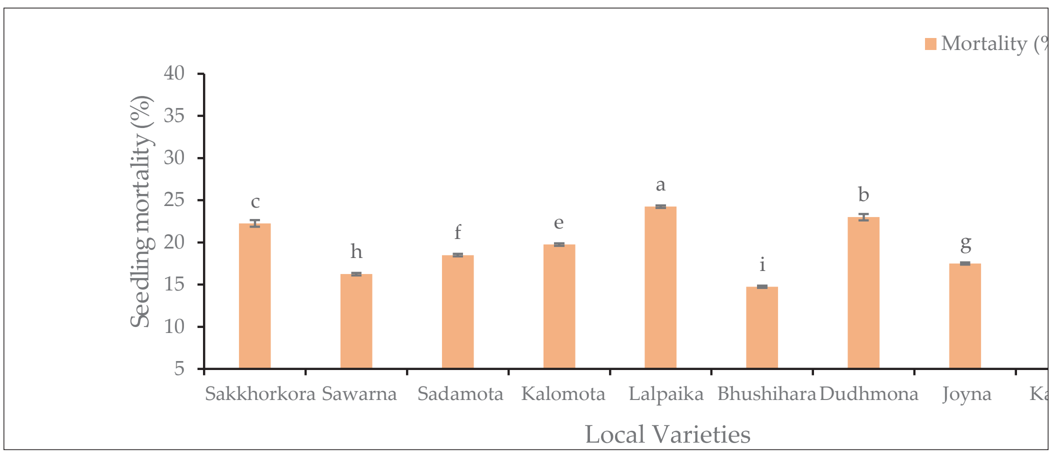


Fig. 5. Seedling mortality of farmers rice seeds of nine locally cultivated varieties. [Bar(s) above which the same letter(s) were not significantly different (P<0.05)].

Several studies examined the germination rate and seedling mortality of rice seeds of different varieties. According to Ora *et al.* (2011), the highest seed germination (99.50%) was observed on Hira-2 and the lowest seed germination (54.63%) was observed in BRRI hybrid dhan-2. They also observed the highest percentage of post-emergence death on Aloron (4.38%) followed by ACI-1 (3.88%) and Modhumoti-2 (3.63%). Ahmed *et al.* (2013) observed that the germination of rice seeds of BR6 was 54.67-85%, while the other two varieties Joya and Pajam showed 58-81.33% and 58-80% germination, respectively. Sultana *et al.* (2018) found the highest 98% germination in BRRI dhan-74 followed by BRRI dhan 70 (96%) and the lowest germination percentage (25%) was observed in BRRI dhan 62. They also observed the highest mortality percentage of rice seedlings in BRRI dhan 62 (16%) followed by BRRI dhan 61 (15.22%) and BRRI dhan 57 (13.34%) and the lowest mortality rate was found in BRRI dhan 74 (2.04%). Previous findings revealed that the percent seed germination and post-emergence mortality of seedlings were directly associated with seed-borne pathogenic infection.

Correlation of total association of F. fujikuroi and F. equiseti with germination and seedling mortality

While analysing the relationship between germination and total infection of *F. fujikuroi* and *F. equiseti*, it was observed that the highest or the lowest seed germination was related to the highest or lowest seed-borne infection of *F. fujikuroi* and *F. equiseti* (Fig. 6) because of the negative correlation between the germination percentage correlation between the germination percentage and total infection of *F. fujikuroi* and *F. equiseti*. Here, the regression line gives a downward sloping curve which indicates that the germination of seeds decreased when the association of *F. fujikuroi* and *F. equiseti* increased or the germination of seeds increased when the occurrence of *F. fujikuroi* and *F. equiseti* decreased.

In the case of seedling mortality, a definite relationship between the mortality of the seedlings and total seed-borne infection of F. fujikuroi and F. equiseti was also observed. While analysing the relationship between seedling mortality and total infection of F. fujikuroi and F. equiseti, it was observed that the highest or the lowest seedling mortality was related to the highest or the lowest infection of seed-borne *F. fujikuroi* and *F. equiseti* (Fig. 7) because of of seed-borne *F. fujikuroi* and *F. equiseti* (Fig. 7) because of positive correlation between the seedling mortality and total infection of *F. fujikuroi* and *F. equiseti*. Here, the regression line gives an upward-sloping curve, revealing that the mortality of the seedlings increases when the occurrence of *F. fujikuroi* and *F. equiseti* increase and decreases when *F. fujikuroi* and *F. equiseti* decrease.

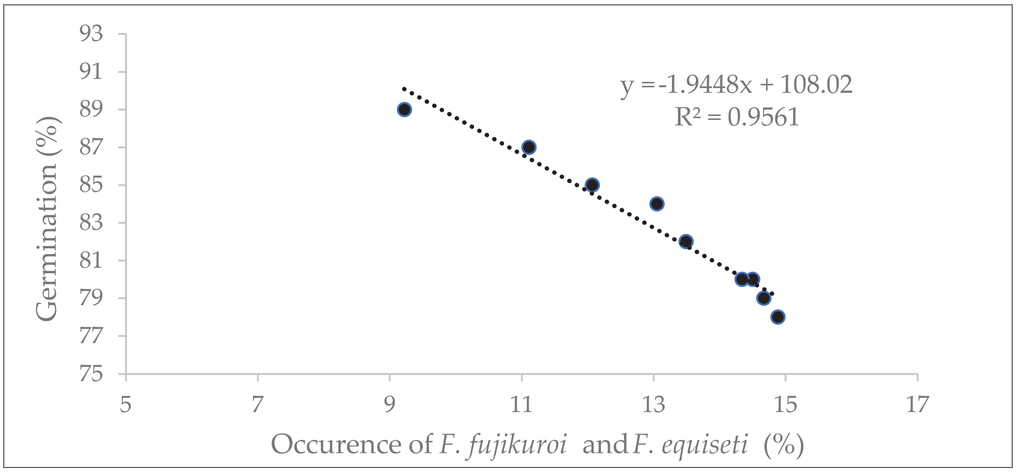


Fig. 6. Relationship between seed germination and total occurrence of *F. fujikuroi* and *F. equiseti* in the collected local varieties.

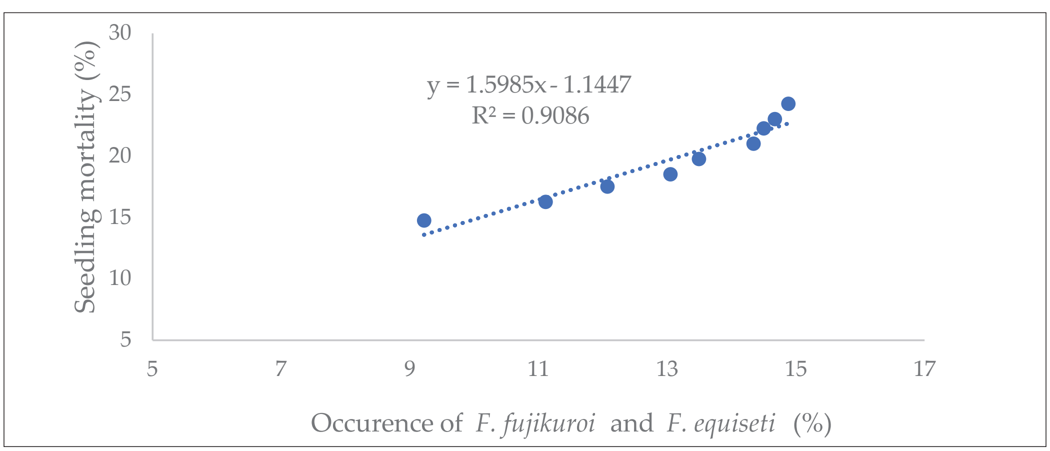


Fig. 7. Relationship between mortality of the seedlings and total association of *F. fujikuroi* and *F. equiseti* in the collected local varieties.

Fungi associated with rice seeds also affect germination and seedling mortality. Sultana *et al.* (2018) observed a relationship between germination rate and seedling mortality with association of seed-borne fungi. The findings of Sultana *et al.* (2018) revealed that mortality of the seedlings decreases when the percentage of fungi decreases or the seedlings mortality increases when the percentage of fungi increases and the rate of germination decreases when the percentage of fungi increases or the germination percentage increases when the percentage of fungi decreases. The findings of Ora *et al.* (2011) also indicated that the germination percentage decreased due to hard and rotten seeds. Rotten seed and post-emergence mortality of seedling were directly associated with seed borne pathogenic infection.

Conclusion

The present study revealed that farmer rice seed samples from all upazila of Barisal district were associated with *F. fujikuroi* and *F. equiseti* and their infection status varied with location and cultivars. Prevalence of these two fungi in certain upazilas and cultivars was markedly higher. Farmers are suggested to use pathogen free seeds or treated seeds with effective fungicides. A further comprehensive study is needed to know the seed health status of other local varieties in other rice-growing areas of Bangladesh.

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