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Incidence of seedborne mycoflora and their effects on germination of maize seeds

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Abstract

A laboratory experiment was conducted with maize seed samples collected from four maize growing districts viz. Bogra, Gaibandha, Rangpur and Mymensingh of Bangladesh following blotter method. Nine species of fungi namely *Aspergillus flavus, A. niger, Alternaria alternata, Bipolaris maydis, Curvularia lunata, Fusarium oxysporum, F. moniliforme, Penicillium oxalicum* and *Rhizopus stolonifer* were identified. Among the species, the incidence of *Aspergillus flavus* was higher (27.42%) followed by *A. niger* (23.47%), *Fusarium oxysporum* (20.55%), *Penicillium oxalicum* (18.37%), *Rhizopus stolonifer* (17.83%), *F. moniliforme* (17.39%), *Curvularia lunata* (7.87%), *Bipolaris maydis* (3.50%) and *Alternaria alternata* (2.74%). The association of the pathogens with maize seeds were observed as deterioration of seed quality and lowered down the germination rate (maximum 78.74% and minimum 67.97%).

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Introduction

Maize (*zea mays* L.) is one of the most important cereal crops in the world and ranks third next to wheat and rice (Aldrich *et al*., 1975). In Bangladesh, it has a good potential as a cereal crop due to its low cost of production, wide adaptability and diversified use. There has been continuous increase in the consumption of corn mainly owning to increase in the demand from meat and starch sector. There is growing requirement of maize from poultry sector where it is being used as feed. It is also fractionated by either dry or wet milling into food and industrial ingredients. Starch, the major constituent of the corn kernel, is used in its native form or after chemical or enzymatic modification, in foods and industrial products. Starch is also converted into glucose or fructose for use as food sweetener. Glucose can be fermented into ethanol for beverages or into many other chemicals. Recently, it has been discovered that corn can also be used in the production of biofuel (Deepavali and Nilima, 2013). However, maize kernels have high nutritive value containing 66.2% starch, 11.1% protein, 7.1% oil and 1.5% minerals. Besides, it contains 90mg carotene, 1.8mg niacin, 0.8mg thiamin and 0.1mg riboflavin per 100g grains (Chowdhury and Islam, 1993).

As seed is the foundation material of agriculture, healthy seeds free from seedborne pathogens are prerequisite for successful crop production. Unfortunately, maize seeds are infected by three major pathogens namely fungi, bacteria and virus and that often reduce both the yield and the quality of grains as well as impair seed germination. As many as 112 diseases are known to occur on corn in the corn growing countries. Of all the diseases, more than 70s are seed borne (USDA, 1960). In Bangladesh, maize suffers from 28 diseases in seedling stage (Bari and Alam, 2004) in which 11 are seedborne in nature (Fakir, 2001).

Some of fungi species which are related to corn mostly belong to *Fusarium* spp. and *Apergillus* spp. There are many reports that indicate these fungi species produce mycotoxin which can be harmful for human health and animals (Gonzalez *et al*., 1995). However, considerable information about seedsborne mycoflora associated with corn is available in some corn producer countries such as USA, Argentina, South America, Canada, China, Pakistan, India etc. (Abbas *et al*., 1988). Fungi affect the quality of grain through increase in fatty acid, reduction in germination, mustiness and finally spoilage of grain. Fungal development in grains is influenced by temperature humidity and period of storage. Survey of literature shows that a number of fungi viz., *Alternaria alternata*, *Aspergillus* spp., *Bipolaris maydis*, *Fusarium moniliforme*, *Fusarium* spp., *Cephalosporium* spp., *Mucor* sp. and *Penicillium* spp., have been reported from maize seed (Tulin, 2006; Ehlam *et al*., 2015). Despite the production of certified seeds of improved varieties of maize, small-scale farmers continue to produce and use their own seeds without knowing the health status of the seed lot. Moreover, very few works have been done in Bangladesh to evaluate the health status of maize seeds from formal and informal seed production sectors. Since seed could play an important role in the epidemics in fields, good quality and healthy seed of maize should be released to farmers in order to secure their outputs. In view of above mentioned facts, the present research work has been undertaken to record the mycoflora associated with maize seeds and to determine the effects of mycoflora on germination of maize seeds.

Materials and methods

The laboratory experiment based on isolation and identification of the causal organisms from collected seed samples was carried out in the Seed Pathology Centre (SPC), Bangladesh Agricultural University (BAU), Mymensingh. The laboratory works were started on 1 April 2008 and completed by 29 April 2008. The experiment was started with six replications following Completely Randomized Design (CRD). The seed samples were collected after harvesting and drying from the five farmers' of each selected districts viz. Bogra, Gaibandha, Rangpur and mymensingh. The seeds of each district collected from five farmers' fields were mixed together to make composite sample.

In case of Mymensingh, seed samples were collected from the GPB field laboratory, BAU, Mymensingh. The seed samples of each district were enclosed individually in paper bags with proper labeling and brought to the SPC and stored in the refrigerator at 5±1 ºC until used for subsequent studies.

Seed health testing followed by blotter method

In this method, three layers of filter papers (Whatman No. 1) were soaked in sterile water and placed at the bottom of 9 cm diameter petridishes. Sixty seeds from each composite sample were taken randomly and then placed on the moist filter paper at the rate of 10 seeds per plate (Fig. 1).

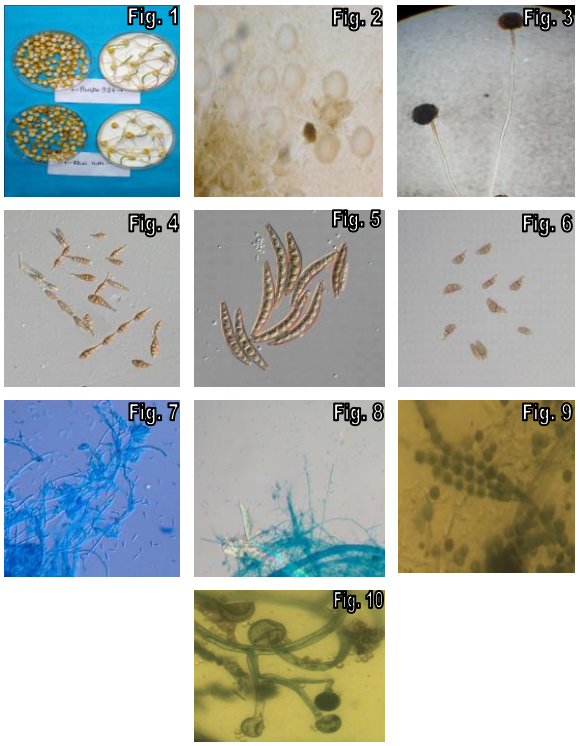

Fig. 1. Germinated maize seeds, **Fig. 2.** Different Structure of *Aspergillus flavus*, **Fig. 3.** *A. niger*, **Fig. 4.** *Alternaria alternata,* **Fig. 5.** *Bipolaris maydis*, **Fig. 6.** *Curvularia lunata*, **Fig. 7.** *Fusarium oxysporum*, **Fig. 8.** *F. moliniforme*, **Fig. 9.** *Penicillium oxalicum* and **Fig. 10.** *Rhizopus stolonifer*.7

Similarly, Two hundred and forty seeds collected from four districts were placed on twenty four petridishes at the same rate.

The petridishes with seeds were then incubated at 20 ± 2 °C under 12/12 alternating cycles of Near Ultra-Violet (NUV) light and darkness in the incubation room of the SPC, BAU, Mymensingh for seven days. Time to time watering was done to keep the filter paper moist. After incubation the seeds were examined under the stereomicroscope for the presence of seed borne fungi. Data were expressed in percentage.

Identification of fungi from the seed samples

Each individual seed was observed under stereomicroscope in order to identify the seed borne fungi. Most of the associated fungi were detected by observing their growth characters on the incubated seeds. The temporary as well as permanent slides were also prepared and observed under compound microscope for proper identification. Photographs of the identified pathogens were taken using Olympus Microscope at 20x magnifications (Field Fertility Clinic, Faculty of Veterinary Science, BAU, Mymensingh). The fungi were identified to species level, wherever possible, following the keys of RamNath *et al.* (1970), Mathur and Kongsdal (2003).

Results

The germination percentage of corn seeds differed significantly from variety to variety and also location to location. Seed germination of corn variety Pacific-984 was significantly higher than the variety Khai Vutta. Seeds collected from Gaibandha showed the highest germination (78.74%) followed by Rangpur (76.15%), Bogra (73.62%) and Mymensingh (67.97%), and per cent seed germination of these three was statistically similar (Table 1).

Table 1. Germination of maize seeds of two varieties collected from four districts.

Variety	District	Per cent Germination
Pacific-984	Rangpur	76.15 b
	Gaibandha	78.74 a
	Bogra	73.62 c
Khai vutta	Mymensingh	67.97 d
CV (%)		1.42
LSD (0.05)		2.11

In a column, figures having common letter (s) do not differ significantly at 5% level by LSD.

Nine species of fungi representing seven genera were identified on corn seeds. The identified fungi were *Aspergillus flavus, A. niger, Alternaria alternata, Bipolaris maydis, Curvualria lunata, Fusarium oxysporum, F. moniliforme, Penicillium oxalicum* and *Rhizopus stolonifer* (Fig. 2, Fig. 3, Fig. 4, Fig. 5, Fig. 6, Fig. 7, Fig. 8, Fig. 9 and Fig. 10). Interaction effect of variety and location indicated that the corn variety Pacific-984 collected from Gaibandha was the best one in terms of seed germination and seed infection as shown in (Table 1, Fig. 1). Considering the fact of per cent seed borne infection by all the fungi species as presented in Table 2, the highest percentage of *Aspergillus flavus* was found in the seeds of the variety khai vutta collected from Mymensingh and it was 27.42 %. The incidence was significantly higher than the others. The lowest incidence was found in Pacific-984 seeds collected from Gaibandha (16.48 %). Insignificant difference was found between Rangpur and Bogra where the per cent seed borne infection levels were 18.48 % and 19.09 % respectively. Per cent seed borne infection by *Aspergillus niger* was higher in Gaibandha (23.47 %) and lower in Mymensingh (13.82 %). Insignificant difference was also found between Rangpur and Bogra where infection levels were 17.52 % and 18.22 % respectively. Per cent seed borne infection by *Alternaria altenata* was not found in the seeds collected from Pangpur and Gaibandha but in Bogra and Mymensingh, where the infection levels were 1.17 % and 2.74 % respectively that was statistically different. Incidence of *Bipolaris maydis* was higher in the seeds of corn variety khai vutta collected from Mymensingh (3.50 %) and lower in Pacific-984 collected from Gaibandha (1.79 %). Statistically similar infection was found among Rangpur (2.52 %), Bogra (3.06 %) and Mymensingh (3.50 %). On the other hand, infection was insignificant between Rangpur and Gaibandha. The highest incidence of *Curvularia lunata* was recorded in the seeds of the variety khai vutta collected from Mymensingh (7.87 %) and the lowest incidence was found in Pacific-984 seeds of Gaibandha (4.6 %). Statistically insignificant difference was found among Rangpur (6.51 %), Gaibandha and Bogra (5.4 %), and also between Rangpur and Mynensingh.

Table 2. Per cent seed borne infection on maize seed samples of two varieties collected from four districts.

Variety	***District***	**Per cent seed borne infection**								
		A. flavus	*A. niger*	*Alternaria alternata*	*Bipolaris maydis*	*Curvularia lunata*	*Fusarium oxysporum*	*F. moniliforme*	*Penicillium oxalicum*	*Rhizopus stolonifer*
Pacific-984	Rangpur	18.48b	17.52b	0.00c	2.52ab	6.51ab	20.55a	17.39a	16.43ab	7.14c
	Gaibandha	16.48c	23.47a	0.00c	1.79b	4.60b	15.25c	9.39d	13.67c	10.64b
	Bogra	19.09b	18.22b	1.17b	3.06a	5.40b	12.84d	12.26c	15.47bc	12.13b
Khai vutta	Mymensingh	27.42a	13.82c	2.74a	3.50a	7.87a	17.76b	15.58b	18.37a	17.83a
CV (%)		1.55	4.49	19.39	19.38	18.18	6.19	6.27	8.13	10.28
LSD (0.05)		0.62	1.63	0.37	1.05	2.21	2.05	1.70	2.74	2.45

In a column, figures having common letter (s) do not differ significantly at 5% level by LSD.

Higher incidence of *Fusarium oxysporum* was found in the seeds of Pacific-984 collected from Rangpur (20.55 %) and lower in Bogra (12.84 %), but statistically insignificant difference was found among the districts. The highest seed borne infection by *F. moniliforme* was recorded in Rangpur (17.39 %) and the lowest in Gaibandha (9.39 %) but infection was statistically significant among the districts. In case of *Penicillium oxalicum*, per cent seed borne infection was higher in Mymensingh (18.37 %) and lower in Rangpur (13.67 %). Statistically insignificant differences were recorded between Rangpur and Bogra (15.47 %), Gaibandha (16.43 %) and Bogra, and Gaibandha and Mymensingh. Finally, per cent seed borne infection by *Rhizopus stolonifer* was the highest in Mymensingh (17.83%) and the lowest in Rangpur (7.14%) which significantly different from others, but insignificant difference was found in Gaibandha and Bogra.

Discussion

The prevalence of fungal infections and seed germination varied depending on corn varieties and sources of seed collection. Significant differences

were observed in the germination percentage of two corn varieties collected from four different districts of Bangladesh. The present experimental results revealed that none of the samples out of twenty had germination higher than 80 % (national standard). This might be due to improper handling and processing of seeds after harvesting, differences in storage condition and kinds of seedborne fungi associated with them. This is in agreement with the observations made by Khan (2006) and Islam (2005).

In the present investigation nine species of fungi viz. *Aspergillus flavus, A. niger, Alternaria alternata, Bipolaris maydis, Curvualria lunata, Fusarium oxysporum, F. moniliforme, Penicillium oxalicum and Rhizopus stolonifer* were detected from maize seeds. These fungi were mostly concentrated in the embryonic region of the seeds resulted in germination failure. In the maize fields, the mature and ripen cobs were found to be infected with different types of fast growing fungi. During grain separation the healthy seeds were also infected by unhealthy one promoted by careless harvesting and processing in open yard.

In the laboratory, *Bipolaris maydis* is reported to be an important seedborne fungus of corn. But fortunately the incidence of *B. maydis* was the lowest among the fungi detected in the present investigation. This might be due to *Bipolaris* free seeds used by the farmers, lower inoculum potential and lower infection of the crop in the farmers' fields. The present findings of the seedborne fungal organisms were in agreement with the information of seedborne nature of the pathogen reported by Sitara and Akhter (2007), Khan (2006), Islam (2005), Owolade *et al.*(2002), Tanaka *et al.*(2001), Fakir (2001), White (1999), Goulart (1993) and Bujari and Earshad (1993).

Conclusion

A total of twenty seed samples collected from different locations of the selected districts after harvesting were studied, and nine species of fungi representing seven genera were identified. The final effects of the pathogens on maize seeds were observed as deterioration of seed quality and lowered down the germination rate.

As the maize got popularity in Bangladesh so, the farmers must pay attention during harvesting and processing of maize and to treat their seeds with proven agro-technologies as well as efforts should be given by government and NGOs in developing and introducing high yielding resistant or least susceptible cultivars to boost up its production.

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