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RESEARCH PAPER

Biochemical Analysis of Maize Seeds as Affected by Seed-Borne Fungi

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ABSTRACT

The experiments were conducted to evaluate the biochemical composition and prevalence of fungi in healthy and infected seeds of three maize cultivars. In laboratory experiment, the germination and prevalence of fungi of three maize cultivars cv. Bornil, BARI Bhutta-5 and BARI Bhutta-6 were investigated by blotter method and six species of fungi representing five genera were identified. Prevalence of seed-borne fungal infections varied significantly depending on the varieties. The identified species were *Aspergillus niger, Aspergillus flavus, Fusarium spp., Curvularia lunata, Penicillium spp.* and *Rhizopus stolonifer*. Of all these fungi, *Fusarium spp.* and *Curvularia lunata* showed the highest and the lowest percent of infection, respectively. Seed germination also varied significantly depending on the varieties. Fungi along with other factors might be responsible for this low germination. Biochemical analysis showed slight changes in proximate value. Protein, fat and moisture content increased while ash content reduced in infected seeds compared to healthy seeds.

Key words: Maize seeds, Fungi, Ash, Chemical reagents, Crude protein, Crude fat

Introduction

Bangladesh is a developing country. Forty-six percent of the population lives below poverty line (BBS, 2010). However, at the present rate of population growth (total as well as per unit area), rice and wheat are unlikely going to be able to meet the total demand of food. Maize is one of the strongest candidates for meeting this demand. Maize is the most important cereal crops in the world, which can be grown round the year even on the marginal land and homestead. It is a good source of carotene. Maize is not only as a major grain crop but also a major source of poultry feed and animal fodder. Furthermore, when dried to 15% moisture maize stover is used as fuel (Annonymous, 1997).

In present situation, maize has a good potential as a cereal crop in Bangladesh for its low cost of production, wide adaptability and diversified use. The average yield of corn in North America is 7.2 t ha^{-1} (FAO, 2006). In Bangladesh, corn has been cultivated in 1, 51,000 ha of land and production was 9, 02,000 t having the average yield 5.9 t/ha during the year 2008-2009 (BBS, 2010). Maize plants are found to be infected by many pathogens and cause various diseases and reduced quality and yield (Anonymous, 1997). As many as 112 diseases are known to occur on maize crops (USDA, 1960). Three major groups of pathogens- fungi, bacteria and viruses can be seed-borne and affect seed health and quality of maize. Among them fungi are predominant. The seed-borne fungal pathogens are *Aspergillus flavus, Aspergillus niger, Rhizopus stolonifer, Alternaria alternate, Curvularia lunata, Helminthosporium turcicum* (Arvinder and Rai, 1991).

Recently, Government has given special emphasis on extensive cultivation and improvement of maize crop in Bangladesh. As a part of this programme, Bangladesh Agricultural Research Institute (BARI) has developed and released a number of maize varieties. Among the released varieties BARI Bhutta-5, BARI Bhutta -6 and Bornali are being extensively cultivated in the country. Though quality healthy seed is the pre-requisite for successful maize production in the country, little is known about the health or occurrence of plant pathogenic fungi, the most important groups of pathogens with the seeds of these promising maize varieties.

Keeping the above points in view, an experiment was conducted to identify the fungi associated with maize seed samples and to determine the quality of maize seeds due to fungal infection.

Materials and methods

Collection and preservation of seed samples: Maize seed samples were collected from farmer's level from Gazipur. Four seed samples for each of the three varieties were obtained from the location. Each sample contained about 200g seeds. Seeds were collected from farmer's house after two to four weeks of harvest during November, 2010. The seed samples were kept in polyethylene bags and stored in the refrigerator of the Seed Pathology Centre at 5±1 ^{0}C.

Laboratory experiment

1. Grading of seed samples: The collected seed samples were sorted out to separate apparently healthy looking and infected seeds.

2. Testing health status of the collected seed samples by Blotter method: Health status of the collected seeds of three popular maize varieties cv. BARI Bhutta -5, BARI Bhutta -6 and Bornali were studied. Sixty plastic petridishes were taken and three presoaked filter papers (Whatman No.1) were placed in each petridish. Two hundred infected and healthy seeds were selected from each seed sample and 10 seeds were placed in each petridish and kept under 12/12 alternating cycles of Near Ultra-Violet (NUV) light and darkness in the incubation room. Germination percentage and incidence of seed yielding of different fungi were recorded with the help of steriobinocular microscope and also by using compound microscope following the keys of Mathur and Kongsdal (1994).

3. Testing of seeds for detecting and identifying prevalence of seed-borne fungi association with maize seed samples: The seeds were assayed for the presence of fungal pathogens by the Standard Blotter Method following the rules of International Seed Testing Association (ISTA, 2001). Each seed was observed under steriobinocular microscope in order to identify the seed-borne fungi. Most of the associated fungi were detected by observing their growth characters on the incubated seeds. Permanent slides were also prepared for pathogen identification and observed under compound microscope. The fungi were identified to species level, wherever possible, following the keys of Malone and Musket (1964). The photographs of the pathogens were taken from the permanent slide.

4. Determination of Proximate Composition

4.1. Moisture: Moisture was estimated according to the method of Pearson (Pearson, 1970). This involves the measurement of the weight loss due to the evaporation of water. Five grams of ground sample was taken in a porcelain crucible, which was previously heated to about 100 ^{0}C and cooled in dessicator followed by weighing. The crucible was then placed in an oven and heated for about five hours at 100˚C. It was then cooled to room temperature in a dessicator and weighed. The process of heating, cooling and weighing were repeated until the weight became constant.

Moisture content (g/100g sample)

$$= \frac{\text{Weight of the moisture}}{\text{Weight of the sample}} \times 100$$

4.2. Total Ash: To determine total ash, 5 g of the sample was taken in a porcelain crucible, which was previously heated to about 100 ^{0}C and cooled in dessicator at room temperature and weighed. The crucible was heated in heater till all material is completely charred, then heated in a muffle furnace for about five hours at 600 ^{0}C. It was then cooled in dessicator and weighed. To ensure completion of ashing, the crucible was again heated in the muffle furnace for half an hour then cooled and weighed again. This step was repeated till two consecutive weights were same and the ash was almost white in colour. The ash content was calculated using following formula.

$$\text{Ash content (g/100g sample)} = \frac{\text{Weight of the ash}}{\text{Weight of the sample}} \times 100$$

4.3. Crude protein: The principle of protein estimation is based on estimating the nitrogen content of the material and then multiplying the nitrogen value by 5.5 or 5.85. This is referred to as crude protein content, since the non-protein nitrogen (NPN) present in the material was taken into consideration in the present investigation. The estimation of nitrogen was made by modified micro-Kjeldahl method (AOAC, 1980), which depends on the fact that organic nitrogen, when digested with concentrated sulphuric acid is converted into ammonium sulphate. Ammonia liberated by making the solution alkaline is distilled into a known volume of standard boric acid, which is then back titrated. Reagents used were; i) Kjel Tab./Catalyst mixture (Potassium sulphate + Selanium), ii) Concentrated sulphuric acid (H_2SO_4) solution, iii) 2 % Boric acid ($HB0_3$) solution, iv) Hydrochloric acid (0.1 N HCl) solution, v) 40 % sodium hydroxide solution (NaOH) and vi) Mixed indicator (Methyl red and Methylene blue).

One hundred milligram (0.l g) of dried ground seed samples was taken in weighing paper and measured accurately. Then it was poured into a 75 ml clean Kjeldahl flask, to which 3 ml conc. H_2SO_4, 1 gel tab., 2 ml H_2O_2 and 2-3 glass boll were added. The sample mixture was heated at 370˚C for 1 hr over a preheated heater. When the sample colour becomes colourless (white) then the digestion of the sample was completed. The digested sample was cooled at room temperature (25 ^{0}C) and dilute to 75 ml. Ten milliliter (10 ml) of the digested dilute sample solution was taken in a distillation apparatus with 10 ml 40 % NaOH. The distillate (about 60 ml) was collected in a conical flask containing 10 ml 2 % Boric acid solution and 2 drops of mixed indicator (methyl red and methylene blue). The total distillate was collected and titrated with standardized HCl solution (0.1 N HCl).

The amount of nitrogen was calculated according to the following equation:

$$\%\ \text{Nitrogen} = \frac{(\text{Ts}-\text{Tb})\times \text{Strengthof HCl}\times 0.014}{\text{Weight of the sample(g)}} \times 100$$

Where,

T_S = Titre value of the sample in ml

T_b = Titre value of the blank in ml

Strength of HCl acid = 0.1 N

Then the % nitrogen of the sample was multiplied by 5.5 or 5.85 to obtain the total crude protein present in the sample according to the equation:

% Crude protein = % Nitrogen x 5.5

4.4. Crude fat or total Lipid: Crude fat was determined with the help of Soxhlet method. Two gram (2 g) of ground whole seed sample were taken in a thimble and extracted continuously with acetone for nearly 20 hours.

The difference in the weight of thimble containing the sample before and after extraction was used to calculate the crude fat.

Statistical analysis: The data were analyzed following the Completely Randomized Design (CRD). The mean differences for efficiency of the treatments were judged by least significant difference (LSD) test.

Results and discussion

The experiments were carried out with maize varieties such as Bornali, BARI Bhutta-5 and BARI Bhutta-6. The results are presented under the following heads.

1. Prevalence of fungi associated with the healthy looking and infected maize seeds

Fungi recorded through blotter method on the healthy looking and infected seeds of maize varieties cv. Bornali, BARI Bhutta-5 and BARI Bhutta-6 are presented in Table 1. Altogether six species of fungi representing five genera were identified on the three selected maize cultivars. The prevalence of *Aspergillus niger, Aspergillus flavus, Fusarium spp., Penicillium spp., Curvularia lunata, Rhizopus stolonifer* were varied significantly with respect to variety. In case of healthy seeds, statistically the highest prevalence of *Aspergillus niger* was recorded in Bornali (18.25%) followed by BARI Bhutta-5 (9.0%), the highest prevalence of *Penicillium spp*. was in Bornali (17%) followed by BARI Bhutta-6 (14%), the highest prevalence of *Fusarium spp*. was in Bornali (12%) followed by BARI Bhutta-5(5%) and BARI Bhutta-6 (5%), the highest prevalence of *Aspergillus flavus* was in Bornali (11%) followed by BARI Bhutta-5 (10%), the highest prevalence of *Rhizopus stolonifer* was in BARI Bhutta-5 (7%) followed by Bornali (6%) BARI Bhutta-6 (6%), the highest prevalence of *Curvularia lunata* was in Bornali (4.5%) followed by BARI Bhutta-6 (2%). In case of infected seeds, statistically the highest prevalence of *Fusarium spp*. was in Bornali (55%) followed by BARI Bhutta-5 (35.50%), the highest prevalence of *Aspergillus niger* was in Bornali (45%) followed by BARI Bhutta-5 (42%), the highest prevalence of *Penicillium spp*. was in Bornali (32%) followed by BARI Bhutta-5 (25%), the highest prevalence of *Aspergillus flavus* was in Bornali (31%) followed by BARI Bhutta-6 (27%), the highest prevalence of *Curvularia lunata* was in Bornali (17%) followed by BARI Bhutta-5 (4%), the highest prevalence of *Rhizopus stolonifer* was in BARI Bhutta-6 (29%) followed by BARI Bhutta-5 (28%).

Table1. Percent seed-borne infection of healthy looking and infected seeds of three maize varieties cv. Bornali, BARI Bhutta-5 and BARI Bhutta-6

Variety	Prevalence of fungi (%)					
	Aspergillus niger		*Aspergillus flavus*		*Curvularia lunata*	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
Bornali	18.25a	45.0 a	11.0 a	31.0 a	4.50 a	17.0 a
BARI Bhutta5	9.00 b	42.0 b	10.0 a	16.0 c	1.50 b	4.0 b
BARI Bhutta6	7.00 c	37.0 c	2.00 b	27.0 b	2.00 b	2.0 c
$LSD_{0.05}$	1.385	2.920	1.686	1.847	0.5985	1.306
CV%	7.59	4.42	13.75	4.68	14.03	10.65

Cont'd

Variety	*Fusarium spp.*		*Penicillium spp.*		*Rhizopus stolonifer*	
	Healthy	Infected	Healthy	Infected	Healthy	Infected
Bornali	12.0 a	55.0 a	17.0 a	32.0 a	6.00 b	10.00 b
BARI Bhutta5	5.0 b	35.5 b	4.00 c	25.0 b	7.00 a	28.00 a
BARI Bhutta6	5.0 b	12.0 c	14.0 b	22.75c	6.00 b	29.00 a
$LSD_{0.05}$	1.306	1.922	1.87	1.577	0.5812	1.31
CV%	11.13	3.52	9.04	3.71	5.73	3.66

2. Effect of the seed-borne fungi on the germination of various maize seeds

The effect of the fungal seed-borne pathogen on germination of healthy and infected seeds of three maize cultivars were studied and presented in Table 2. The effect of fungal pathogens associated with various maize seeds significantly differ from cultivar to cultivar. The highest germination was recorded in BARI Bhutta-6 (98%) and the lowest germination was recorded in Bornali (91.50%). Among the infected seeds of three maize varieties the highest germination was recorded in BARI Bhutta-6 (24%) and the lowest in Bornali (12.25%). Statistically the highest effect of the fungal prevalence reflected the lowest germination as recorded in the infected seeds of Bornali (12.25%) while significantly the highest germination of infected was recorded in the infected seeds of BARI Bhutta-6 (24%).

Fakir (2001) reported 11 seed-borne diseases occurring on maize in Bangladesh of which

Aspergillus spp. is responsible for germination failure. Sultana (2009) reported that, in the blotter test, the species of Fusarium (*Fusarium moniliforme* and *Fusarium oxysporum*) and *A. flavus* were always found to be associated with the ungerminated seeds. These information about the effect of seed-borne fungal organism on germination are in agreement with the present findings.

Table 2. Germination of maize varieties in blotter method (based on 400 Seeds)

Variety	Seed Germination (%)	
	Healthy	Infected
Bornali	91.50 b	12.25 c
BARI Bhutta-5	95.00 a b	15.00 b
BARI Bhutta-6	98.00 a	24.00 a
$LSD_{0.05}$	4.679	1.748
CV%	3.08	6.40

3. Effect of the seed-borne fungi on protein content of three Maize varieties

The protein content of healthy looking seeds of three maize varieties was found to range between 8.04% and 10.70% (Table 3). Among the healthy looking seeds of three maize varieties, the highest amount of protein was recorded in BARI Bhutta-6 (10.70%) while the lowest amount of protein was recorded in Bornali (8.04%). The protein content of infected seeds of three maize varieties was found to range between 8.71% and 11.88% (Table 3). Among the infected seeds of three maize varieties, the highest amount of protein was recorded in BARI Bhutta-6 (11.88%) while the lowest amount of protein was recorded in BARI Bhutta-5 (8.71%).

The present findings clearly showed that protein contents of infected maize seeds are higher than the protein contents of healthy looking seeds of three maize varieties which is in agreement with the information reported by B. Kashinath *et al.* (2002).

Table 3. Moisture, ash, protein, fat content of healthy looking and infected seeds of three maize varieties.

Variety	Moisture content (gm)		Ash content (gm)		Protein content (gm)		Fat content (gm)	
	Healthy	Infectd	Healthy	Infected	Healthy	Infected	Healthy	Infected
Bornali	14.91 a	15.45 a	1.920 a	1.460 a	10.04 a	10.52 b	3.04 b	3.09 b
BARI Bhutta-5	14.37 a	14.89 a	1.830 b	1.260 b	8.040 b	8.715 c	3.07 a b	3.09 b
BARI Bhutta- 6	12.87 b	13.39 b	1.925 a	1.350 ab	10.70 a	11.88 a	3.11 a	3.145 a
$LSD_{0.05}$	0.795	0.7787	0.08761	0.1431	0.699	0.7486	0.05058	0.05058
CV%	3.54	3.34	2.67	6.58	4.55	4.51	1.03	1.93

4. Effect of the seed-borne fungi on fat content of three maize Varieties

The fat content of healthy looking seeds of three maize varieties was found to range between 3.04% and 3.11% (Table 3). Among the healthy looking seeds of three maize varieties, the highest amount of fat was recorded in BARI Bhutta-6 (3.11%) while the lowest amount of fat was recorded in Bornali (3.04%). The fat content of infected seeds of three maize varieties was found to range between 3.09 and 3.14 (Table 3). Among the infected seeds of three maize varieties, the highest amount of fat was recorded in BARI Bhutta-6 (3.14%) while the lowest amount of fat was recorded in Bornali (3.09%).

The present findings clearly showed that fat contents of infected maize seeds are higher than the fat contents of healthy looking seeds of three maize varieties which is in agreement with the information reported by B. Kashinath *et al.* (2002).

5. Effect of the seed-borne fungi on ash content of three Maize varieties

The ash content of healthy looking seeds of three maize varieties was found to range between 1.830% and 1.925% (Table 3). Among the healthy looking seeds of three maize varieties, the highest amount of ash was recorded in Bornali (1.925 %) while the lowest amount of ash was recorded in BARI Bhutta-5 (1.830 %). The ash content of infected seeds of three maize varieties was found to range between 1.26% and 1.46% (Table 3). Among the infected seeds of three maize varieties, the highest amount of ash was recorded in BARI Bhutta-6 (1.46%) while the lowest amount of ash was recorded in BARI Bhutta-5 (1.26%).

The present findings clearly showed that ash contents of infected maize seeds are less than the ash contents of healthy looking seeds of three maize varieties. There is no report on the variation in the ash content between infected maize seed and healthy maize seed. However, such variation in the ash content between infected seed and healthy seed has been demonstrated in wheat (Malaker *et al.* 2002).

6. Effect of the seed-borne fungi on moisture content of three Maize varieties

The moisture content of healthy looking seeds of three maize varieties was found to range between 12.87 % and 14.91% (Table 3). Among the healthy looking seeds of three maize varieties, the highest amount of moisture was recorded in Bornali (14.91%) while the lowest amount of moisture was recorded in BARI Bhutta-6 (12.87%). The moisture content of infected seeds of three maize varieties was found to range between 13.39% and 15.45% (Table 3). Among the infected seeds of three maize varieties, the highest amount of moisture was recorded in Bornali (15.45 %) while the lowest amount of moisture was recorded in BARI Bhutta-6 (13.39%).

The present findings clearly showed that moisture contents of infected maize seeds are higher than the moisture contents of healthy looking seeds of three maize varieties. There is no report on the variation in the moisture content between infected maize seed and healthy maize seed. However, such variation in the moisture content between infected seed and healthy seed has been demonstrated in wheat.

Summary and Conclusion

We can summarize that highest fungal infection was recorded in the infected seeds of Bornali while the lowest fungal infection was recorded in the healthy seeds. Low germination encountered in the seeds produced by the farmers might be due to the collective influence of the total population of fungi along with other factors. Little variation was found in moisture content between the healthy seeds and infected seeds of respective variety. A little variation was found in ash content between the healthy seeds and infected seeds of respective variety. Ash content of infected maize seeds was lower than that of healthy looking maize seeds. In respect of protein content of the varieties under study, a little variation was found in protein content between the healthy seeds and infected seeds of respective variety. Protein content of infected maize seeds was higher than that of healthy looking maize seeds. In respect of fat content of the varieties under study, a little variation was found in fat content between the healthy seeds and infected seeds of respective variety.

Finally we can conclude that health and biochemical analysis of maize seeds shows that prevalence of fungi in infected seeds causes germination failure and biochemical changes in proximate value.

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