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Probability of mycotoxin contamination during post harvest operations of boro paddy

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Abstract

John Deere and Frugal Moisture meter were compared with that of Indosaw standard. Frugal moisture meter was similar to the Indosaw standard but John Deere moisture meter were, on average, 2% higher than the standard. It is believed the John Deere model can be recalibrated to provide accurate results. Baseline survey on mycotoxins among 200 households of two districts of Bangladesh through the questionnaire indicate that the farmers were familiar with grain discoloration due to mold contamination but they did not have any idea about mycotoxins production on grains. Grain samples, one kg from each of 200 households were collected during May and June (Boro 2015), 119 had moisture content >14% and 37 had moisture content >20%. Purity, discoloration, mold invasion, insects and germination were assessed in the laboratory. Inert matter was 10% in 142 samples and >15% in 15 samples. Molds were associated with grain discoloration. Samples with high moisture content had higher quantity of moldy grains and stored pests. There was 25% grain discoloration of which 18% were moldy when the moisture content was >20%. Blotter incubation test revealed 19% incidence of *Aspergillus* growth on the 19% of the grains and *Fusarium* growth in 9% of the grains and produce Aflatoxins produced by *Aspergillis* and fumonisins by *Fusarium* were detected in rough rice of India (Reddy *et al.,* 1986). Detection and quantification has of these two mycotoxins have not yet been done in Bangladesh. Through the present study the paddy samples will be analyzed by USDA Romer Lab test kit strips to create a national database on mycotoxins in paddy of Bangladesh.

Keywords: Molds, Mycotoxins, Paddy, *Aspergillus, Fusarium*

Introduction

Paddy is a major food crop in Bangladesh. Humid and warm climate associated with frequent rainfall during harvest and storage of moist seeds in open containers results in deterioration of grain quality due to growth of molds (Majiwa, 2007). Most common molds that grow on paddy are *Aspergillus spp, Fusarium spp, Penicillium spp, Curvularia spp, Rhizopus spp, etc.* Among them *Aspergillus spp and Fusarium spp are* frequent in occurrence. *Aspergillus spp and Fusarium spp are* known to produce Aflatoxin and Fumonisin, respectively (David *et al.,* 2004).

Concentration of Aflatoxin and Fumonisin depends on the growth of the molds on the grains which is favored by high moisture content of the grains, hot and humid weather along with rainfall. There are reports of association of Aflatoxin contaminations with *Aspergillus flavus* in whole grain rice and brown rice in 110 samples collected from different parts of Liaoning Province of North-East China (Liu *et. al., 2005*).

Attack of stored pests during harvest and storage enhance molds growth on the grains. This situation is aggravated in places where harvest season coincides with the wet months since most farmers rely on sun drying to reduce the moisture content of freshly harvested grains (Ilangantileke, 1987).

Molds produce mycotoxins on stored grains as secondary metabolites stored grain. Two of the most important mycotoxins are the Aflatoxins and the Fumonisins. Aflatoxins are synthesized by various species of *Aspergillus*, primarily *Aspergillus flavus* and *Aspergillus parasiticus*. Fumonisins are synthesized by various species of *Fusarium*, primarily *Fusarium verticillioides* and *Fusarium proliferatum* (Bilgrami and Sinha, 1986). These mycotoxins are carcinogenic, toxic to humans and domesticated animals, and heat stable in most foodstuffs. Aflatoxins have been reported from the paddy growing areas of India, Vietnam, Japan and the Philippines.

Mycotoxin contamination in paddy is a problem paddy for storage with ≥ 12% moisture content. Post-harvest losses of food grains, caused by insect infestation and mold activity, have been conservatively estimated at 10–15% (Grolleaud, 2002). The storage molds like *Aspergillus candidus*, *A. flavus*, *A. fumigatus*, *A. niger*, *A. versicolor*, *Chaetomium globosum,* and *Penicillium citrinum* were detected in milled rice in Malaysia (Udagawa, 1976). *Aspergillus .flavus*, *A. sydowi*, *A. terreus*, *A. fumigatus*, *A. ochraceus*, *Penicillium corylophilum*, *P. chrysogenum Fusarium. oxysporum*, *Alternaria tenuis*, *Cladosporium cladosporioides*, *Trichoderma viride*, and *Mucor racemosus* were identified in 64 paddy samples from Egypt Hafez *et al.,* 2004). Stored rice grains were milled after a period of storage. Grain parts were separated and estimated the level of contamination with *Aspergillus sp*. The external and internal growths of *Aspergillus* in parts of the grains were observed (Reddy *et al.,* 2006).

Paddy seeds in Thailand contained high levels of incidence of a large number of fungi, while milled rice contained very few fungal contaminants (Pitt et al., 1994). High incidence of *A. flavus* was found rice bran (Elangovan *et al.,* 1999). Eleven *Fusarium* sp. were isolated from paddy seed samples collected from the fields at the foothills of the Himalayas in Nepal (Desjardins *et al.,* 2000).

Consumption of molded grains introduces mycotoxins; even at low concentration reduce growth and development of the children. Mold infection also reduces the market price of the grains, causing economic loss. In addition, molds cause germination failure and hence reduce planting value and price of paddy as planting material. There has been no systematic research in Bangladesh for mycotoxins contamination in paddy. It is a national need to develop a dedicated mycotoxins lab and test the paddy samples for detection and quantification of mycotoxins in paddy. Therefore, the present study was undertaken to study the possibility of mycotoxins contamination on paddy during post harvest operations of Boro paddy in Bangladesh

Materials and Methods

Two hundred farm households were selected four villages of Phulpur upazila of Mymensingh district and four villages of Monirampur upazila of Jessore district of Bangladesh. The guidelines of the USAID project "Feed the Future Post Harvest Loss Reduction Lab (PHLIL)" was followed for farm house hold selection. A total of 200 submitted samples of grains, @ one sample/farm house hold were collected. The samples were taken to the Mycotoxins Lab, BAU and preserved for further study. Moisture contents of the 200 paddy samples were determined by the USDA John Deere digital moisture meter following the instruction on the Guide Book.

PHLIL has donated two types of digital moisture meters to the Mycotoxins Lab-BAU. The accuracy of John Deere and Frugal Sensor moisture meter was compared with that of Indosaw standard moisture meter. Five samples were taken from each of BADC and Ispahani Seed Company. Moisture contents were determined by the three moisture meters.

Grains of 70 gm from each sample was separated into three fractions i) Pure seeds ii) other seeds iii) inert matter. Weight of individual components was taken up to 2 decimal of fraction. An electrical balance was used. Percentage of each component was calculated. Dry inspection was done according to ISTA procedure. Grains, 100 gm from each sample was separated in to three categories as spotted, discolored and moldy. Working sample was prepared by conical seed divider from the submitted samples. Amount of insect damage grains was estimated.

Four hundred seeds were taken randomly from each sample and tested for germination in sand (ISTA, 1976). The seeds were exposed to the room temperature (25-30^{o}) and relative humidity 70-80%. Counting was done at 10 days after seeds setting. The number of normal seedlings, and abnormal seedlings, dead seeds and un-germinated seeds were counted. Results were expressed as percentage.

Three-layers of sterile & moist blotting papers were placed at the bottom of 9 cm diameter plastic Petri dish, 25 seeds were placed on the blotting paper in each Petri dish. Four hundred seeds were incubated at 25^{0}c ± 1^{0}c under 12 hour's cycle of alternate Near Ultra Violet (NUV) light and darkness. Individual seed was observed after 5 days of incubation. The fungal colonies were identified under compound microscope. Seed- borne infection was recorded and expressed in percentage (Agarwal, V.K. and O.V. Singh 1974).

Results and Discussion

Rapid, accurate, easy, economic and portable device is essential for determination of moisture content of the grains critical to assessing rice quality. The John Deere and Frugal Moisture meters were compared with that of Indosaw standard. These devices are economic and portable. The Frugal moisture meter was similar to the Indosaw standard. The readings of John Deere moisture meter were, on average, 2% higher than the standard. It is believed the John Deere model can be recalibrated to provide accurate results (Table 1).

Table 1. Comparison of John Deere and Frugal Sensor with Indosaw moisture meter

Sample/lot	Moisture content (%)		
	Jhon Deere	Frugal	Indosent
BADC/15/01	14.80	12.67	12.43
BADC/15/02	14.60	12.75	12.60
BADC/15/03	14.50	12.51	12.40
BADC/15/04	14.50	12.56	12.20
BADC/15/05	14.70	12.34	12.30
IS/Rang/15/301	15.80	13.77	13.40
IS/Rang/15/302	15.60	13.79	13.10
IS/Rang/15/303	15.50	13.61	13.30
IS/Rang/15/304	15.50	13.60	13.20
IS/Rang/15/305	15.70	13.74	13.30

There was variation in moisture content of the grains of the farm house holds.

Two hundred samples were tested for moisture content of the grains, 119 samples had moisture content (MC) more than 14% and 37 samples had moisture content more than 20% (Table 2). About 44 farmer's samples had moisture content within 10-12% and insect damage was 2.1% in July and 3.5% in October. Another 37 samples had moisture content higher than 12% but less than 14% and insect damage was 5.2% in July and 6.5% in October. Insect damage was as high as 15.5% in the samples with moisture content more than 20% in October. The result in Table 2 clearly indicates that insect damage was higher in the farmer lots which had high moisture content in the grains.

Table 2. Moisture content of the grain and insect damage

Range of MC (%)	Nos. of samples	Damage	
		July	October
10- 12	44	2.1	3.5
12.1-14	37	5.2	6.5
14.1-16	61	7.6	9.3
16.1-18	14	9.3	11.2
18.1-20	07	10.2	12.5
>20	30	13.4	15.5

Inert matter contains high moisture and inocula of storage molds. Percentage of inert matter of the 200 samples were assessed in the laboratory, each sample was separated by purity analysis into pure grains and inert matter. Inert matter was 5-10% in 142 samples and 11-15% in 19 samples. Ten samples had 16-25% inert matter (Table 3).

Table 3. Purity of the farmers samples of paddy

Range of Purity (%)	Inert matter Range (%)	Nos. of samples
96- 98	2-4	29
91-95	5-10	142
84-90	11-15	19
75-85	16-25	10
Total		200

Molds linked to mycotoxin formation causes discoloration on the surface of grains (Arunrat *et al.,* 1981). The percentage of moldy grains was higher in the samples which had higher moisture content. There was 25.1% moldy grain in October when the moisture content was >20% as compared to 21.1% in case of the grains with 18.1-20% moisture content. There were 3.1% moldy grains in July and 7.3% in October in the lot having 12% moisture or less than 12% moisture in the grains (Table 4).

Table 4. Percentage of moldy grains in the farmer storage

Range of MC (%)	Nos. of samples	Moldy grains	
		July	October
10- 12	44	3.1	7.3
12.1-14	37	4.2	11.3
14.1-16	61	5.2	15.5
16.1-18	14	6.3	21.2
18.1-20	07	7.5	21.1
>20	30	8.4	25.1

Molds on the discolored grains grow under favorable conditions. Four hundred discolored seeds were taken at random from each sample having >18% moisture content and tested by Blotter incubation method for mold incidence. *Aspergillus* growth was observed in 19% of the grains in October as compared to 10% in July and 1% in May. *Fusarium* growth was observed in 9% of the grains in October and 3% in July and 1% in May. Other molds such as *Penicillium* were associated with 3% grains in October and in 1% grains in July. *Rhizopu*s growth was observed in 10% grains in October and in 5% grains in July. The molds *Aspergillus* spp are known to produce Aflatoxin and contaminate food grains particularly rice (Pallavi *et al.,* 1997). Fumonisin, other mycotoxins are known to produce in cereals by the molds *Fusarium* (Table 5).

Table 5. Percentage of moldy grains in the farmer storage

Molds	Molds growth on grains		
	May	July	October
Aspergillus spp	1	10	19
Fusarium spp	1	3	9
Penicillum spp	0	1	3
Rhizopus spp	5	7	10
Other spp	3	5	9

High moisture content of the grains enhance molds invasion are reported to cause germination failure (Rahman *et al.,* 1997*)*. Samples having moisture content >18% were tested and found 38-43% germination in October as compared to 94% in the samples with moisture content <12% (Table 5). Germination was 55% in the farmer's lots in October which was 95% in July where moisture content was 16-18% in the grains. There was consistent decrease in germination with the subsequent increase in moisture content in the grains.

Table 6. Germination of grains stored in farmers houses

Molds	Germination (%)		
	May	July	October
10- 12	43	95	94
12.1-14	43	94	87
14.1-16	42	96	78
16.1-18	41	95	55
18.1-20	42	94	43
>20	42	94	38

Farmer's perception about the mycotoxins contamination in paddy was assessed by a questionnaire among 200 farm households in the study area (Table 7). The farmers were familiar with mold and grain discoloration in their store after 2-3 months. They narrated that molds and discoloration were common in their stored grains. They do not have idea about its impact on health. They have no idea about mycotoxins.

Table 7. Farmers' perception about molds and mycotoxins contamination in paddy

Sl. No.	Item	Perception
1.	Moldy grains Grain discoloration	Some external growth on the grain surface are visible Discolored grains surface are visible
2	Detection of molds	Black, pale green, brown blue white visible after 2-3 month of storage.
3	Cause of molds	Grains stored in their traditional containers. Rainfall during storage period. Cloudy sky during drying period. No facility for drying.
4	Toxin contamination	They do not have any idea about mycotoxin contamination in their grains due to high moisture content.
5	Toxicity	They do not have any idea about carcinogenic toxicity in feeding toxin contaminated grains.
6	Control of molds	They do not have idea how to control the molds and toxin contamination

Molds growing on grains present a threat through production of mycotoxins, the secondary metabolites produced by fungi that grow on a wide range of agricultural commodities including cereals and oilseeds (Epstein et al., 1970). Mycotoxins pose a serious health risk to both humans and animals (Van Rosenburg, 1977; Vedman, 2004). *Fusarium proliferatem was* detected from unpolished rice (Abbas *et al.,* 1997, 1999). Makun *et al.,* (2007) found *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, *Mucor*, *Rhizopus*, *Trichoderma*, *Curvularia*, *Helminthosporium*, and *Cladosporiam*in in 196 moldy rice samples in Nigeria.

Paddy seeds in Thailand contained high levels of incidence of a large number of fungi, while milled rice contained very few fungal contaminants (Pitt et al., 1994). High incidence of *A. flavus* was found in rice bran (Elangovan *et al.,* 1999). Eleven *Fusarium* sp. were isolated from paddy seed samples collected from the fields at the foothills of the Himalayas in Nepal (Desjardins *et al.,* 2000).

The incidences of *Aspergillus flavus* colonies in broken rice grains ranges from 11 to 12% in India. Aflatoxin was detected in raw rice in the range of 20 to 98 ppb in 2% samples out of a total of 364 samples. In another study, it was reported to have 3% of the paddy samples contaminated with Aflatoxin at levels up to 600 ppb; 10% samples had Aflatoxins up to 180 ppb and 1% samples contained Aflatoxin at concentrations up to 71 ppb (Reddy *et al.,* 1986). In the Philippines, milled or brown rice when inoculated with toxicogenic strains of *Aspergillus flavus* and *Aspergillus parasiticus*, yielded very high levels of toxin. Aflatoxin B1 in brown rice was found to be concentrated on the bran layers whereas the polished rice contained no toxin or only traces of Aflatoxin (Ilag, 1984).

Conclusion

Grain samples of the farmers of Bangladesh had high moisture content that enhanced the growth of molds. The farmer samples collected from 200 farm households of Bangladesh with high moisture content had high amount of discolored grains, insert matter and insects. Grains are mostly susceptible to mycotoxins production because they are sources of carbohydrates, or sugars. The nutrients of food product invite risk of contamination by the mold fungi. These fungi produce secondary metabolites, mycotoxins. Consumption of low mold infected grains not only toxic but can cause mal-nutrition due deterioration of nutrient value. There has been no research about detection and quantification of the mycotoxins in rough grains of paddy samples of the farmers in Bangladesh. Thus, the paddy samples will be analyzed by Romer lab kit strips for detection and quantification of Aflatoxins and Fumonisin as well nutrient status of the mold infected grains will be determined by biochemical test. Dedicated facilities will be established to develop a National Mycotoxins Lab in Bangladesh.

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