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RESEARCH ARTICLE

ASSESSMENT OF AFLATOXIN PRODUCING FUNGI IN STORED WHEAT (TRITICUMAESTIVUM L.)

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ABSTRACT

The aim of this research work was to assess the aflatoxin contamination of wheat during the storage time therefore wheat samples were collected with different time intervals i-e May, July and October 2014 from upper, central and bottom sack of the same selected stack from 11 public godowns of Hyderabad Division, for Mycological study of aflatoxin in stored wheat stock samples. The results revealed that percentage frequency of aflatoxin producing fungi (*Aspergillus parasiticus and Aspergillus flavus*) was found increased in the bottom portion during the month of October in comparison to upper and central portions of the months May and July, and higher concentration of afllatoxin were found in the higher frequency percentile samples that is Bolhari, Dadu, Aarazigodowns showed 22ng/g concentration of aflatoxin whereas K.N shah and Sehwan having concentration of about 23ng/g, the percentage of moisture in highly contaminated wheat samples were also noted high i-e 13.7, 14.8, 14.9, 14.5 and 14.6% respectively. In proximate findings no such effect was seen in crude fiber and fat but protein (11.99%) and moisture (8.56%) showed lower value at the bottom sack wheat in the samples that showed higher contamination. Wet and dry gluten was found low in all portions of sacks but among all portions the bottom sacks showed more decreased i-e 22.11% in wet and 9.67% in dry gluten due to high contamination in wheat samples taken in the month of October. It was concluded that Aflatoxin producing fungi develop their colonies along with increasing storage time in godowns due to moisture, temperature and change of weather such as monsoon, which was observed the most favorable condition for growing their colonies contaminating the wheat and making it unsuitable for human consumption.

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INTRODUCTION

Wheat (*Triticumaestivum*) is fundamental source of food for 35% world population(USDA) and is the most staple food of people of Pakistan, being agrarian country Pakistan earns 35-40% national income from wheat (GOP 2006), but 20% of the wheat lost every year due to diseases which would be available for food and feed purpose (Fakir, 1999). The major cause of this lose are the seed born fungi called 'Mycotoxin' which are low molecular and are chemically diversified compound produced as secondary metabolite (Anonymous, 1991). Poor harvesting practices and improper storage can contribute to fungal growth and increase the risk of mycotoxin production, resulting contamination of foods and feeds and become major concern in human and livestock poisoning (Wagacha and Muthomi, 2008). About 300 mycotoxins have been recognized, but the scientific community have focused mainly on those that are known as carcinogenic and/or toxic (Zain, 2011). Among all mycotoxin the naturally produced aflatoxins are one of the most potent mycotoxin which can contaminate the wheat (Maliha, 2010). The International Agency for Research on Cancer has classified naturally-occurring mixtures of aflatoxins as carcinogenic to humans in group 1, (Salem and Ahmad, 2010) and these toxins are environmentally stable as resistant towards thermal changes (Scudamore, 2005). These toxins can cause problem in grain storage, because of many important factors like due to previously existent conditions such as moisture content, temperature, storage period, contamination

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rate, broken grain and impurities, insect presence, oxygen rate, damages during harvest processing and grain and seed transport (Lazzari, 1997; Scussel, 2002; Santos, 2002; Garcia *et al.,* 2003; Scudamore, 2005). Therefore present study has been planned to assess the increased colonization of aflatoxin producing fungi and their contamination to wheat during the storage along with the assessment of chemical properties of grain caused by mold fungi, that is why the wheat godown has been chosen for study because at Pakistan godownsmostly used for wheat storage.

MATERIALS AND METHODS

After harvesting of wheat crop in March 2014 the sampling was done in different time intervals of May, July and October from all Government godown of Hyderabad Division (Bolhari. Hali road, Fatah chowk, Hala city, Matiyaari, Thatta, Tandpallhyaar, Dadu, K.N shah, SehwanAarazi), from the upper, central and bottom portion sacksof selected stack, temperature and relative humidity of all selected godowns were recorded i-e 37-52°C and 40-70% respectively, also the monsoon rainfall was consider (mili meters were not measured)in the study, and observed heavy rainfall from August upto start of September month in areas of Dadu, K.N shah and Sehwan, light rainfall were noted in the Thatta (Makli). All the wheat samples were taken to Cereal technology laboratory, institute of Food Sciences and Technology, SAU, Tandojam for analysis, some of the grain was passed from PertenInframatic Grain Analyzer (9200) without any treatment to check the grain moisture% and some of the grain was used for the mycological study whereas some part of thecontaminated wheat grain samples was used for proximate analysis.

Mycological Study

Mycological studies was conducted to determine Mycoflora associated wheat grain samples through Agar plate (PDA) by procedure described by Mathur *et al*. (2003). The relative isolation frequency (Fq.) of aflatoxin producing fungi (*Aspergillus parasiticus and Aspargillusflavus)* calculated as recorded by Fatma Bensassi *et al*. (2011).

Enzyme linked immunosorbent assay (ELISA) technique was used for quantitative analysis for this the commercial immunoassay kit i-e Neogen ELISA Kit (Veratox , Product no. 8030) based on competitive direct enzyme linked immunosorbent assay format were used. Wheat samples was ground and 25ml of 70% methanol was added and filtered through whatman no. 1 filter paper then this filtrate was used for further analysis.Concentration was calculated by ELISA 'state fax 2100' (Awareness technology).

Proximate Study

For assessment of changes in some chemical properties of wheat grains caused by some mold fungi, the highly contaminated wheat grains of October month was taken and wheat was milled using Barbender Quadrametic junior mill, Model Number: 88013.001Germany, for obtaining whole wheat flour, then whole wheat flour was used for analysis of moisture, protein, fat, crude fiber, wet and dry gluten contents, according to standard methods (AACC, 2000).

Data Analysis

All the experiments had three replicates. Data was analyzed for one-way analysis of variance followed by Student-Newman-Keuls multiple testat 0.05 level using compare means procedure of SPSS 16.

RESULTS AND DISCUSSION

Aflatoxin is a problem in many commodities therefore examination of wheat quality is of great importance in food and feed, after harvest when wheat stored in godowns the fungi become colonize under favorable conditions due to improper storage, produce certain type of mycotoxin.

Data revealed in Figure 1 that frequency percentage of aflatoxin producing fungi i-e *Aspargillusparasiticus* and *Aspargillusflavus* were calculated in different time intervals of May, July and October and higher frequency percentage was found in the month of October the *A. parasiticus* was recorded 28.45% and *A. flavus* having 10.33% frequency percent during storage time.

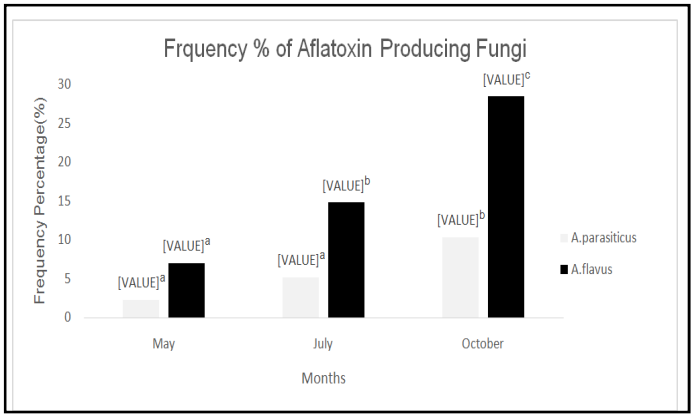
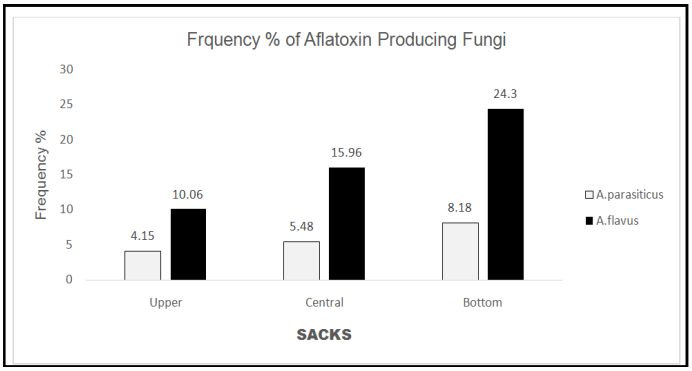


Figure 1. Average of Frequency % of Aflatoxin producing Fungi in different sampling months

Aspergillus parasiticus (F= 11.858, P=0.000, df = 98); *Aspergillus flavus* (F= 36.540, P=0.00, df = 98)

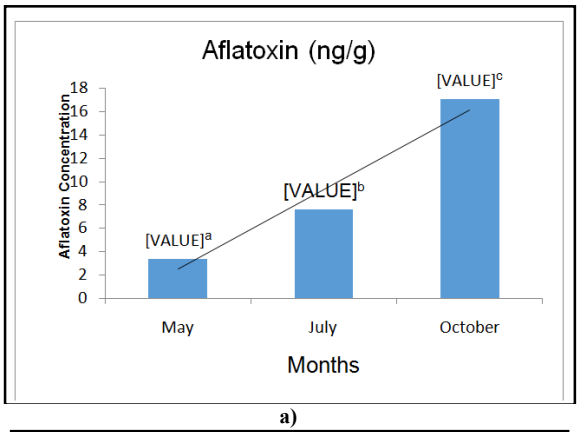
The present results are in consistent with previously reported data that 96.7% wheat was contaminated with *Aspergillus spp*. in the storage period of 180 days after being harvested (Birck *et al*., 2003). Stored peanuts from Madurai godown were highly contaminated with aflatoxin producing fungi *A. flavus* (Rajarajan *et al.,* 2013) studied.

Results in the Figure 2 showed that among the every sampling time from selected stack the bottom portion gives higher percentage of frequency of *A. parasiticus* and *A. Flavus* as the aflatoxin producing fungi get favorable condition therefore it is said that the portion matters in the storage, wheat of middle portion of silos produce more aflatoxin producing fungi (Birck *et al*., 2003).

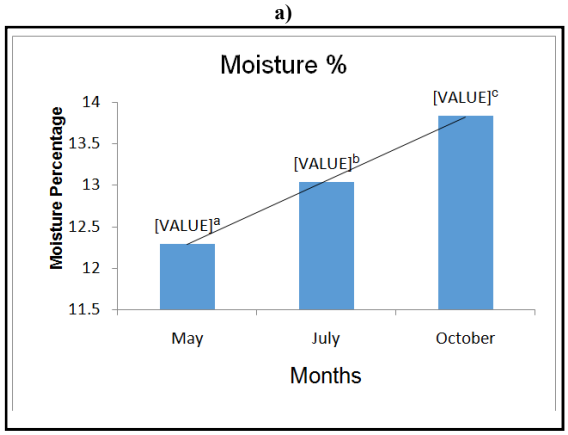


Aspergillus parasiticus (F= 2.536, P=0.084, df = 98); *Aspergillus flavus* (F= 11.161, P=0.00, df = 98)

Figure 2. Average of Frequency % of Aflatoxin Producing fungi in different portions of Sacks



a)



b)

(a)Aflatoxin ng/g (F= 106.758, P=0.00, df = 98); (b)Moisture Content (F= 64.572, P= 0.00, df =98)

Figure 3. (a) Aflatoxin Concentration in wheat; (b) Moisture content of Wheat Grain

Aflatoxin concentration during storage depends on the grain moisture percentage. Results in the Figure 3 and 4 revealing that higher the moisture higher the concentration of aflatoxin was noted, therefore the wheat samples in the month of May showed lower moisture % because May is consider the hottest month at the Sindh, due to the higher temperature the humidity becomes low which directly affects grain moisture percent therefore moisture (%) was found low in Bolhari, Matiyaari and Maklii-e 12.65, 10.9, and 12.5% respectively and giving concentration of 0ng/g at the upper portion sack wheat, whereas after monsoon rainfall the moisture percentage of Dadu (14.8%), K.N Shah (14.5%), Sehwan (14.9%) and Aarazi (14.6%) increased due to which aflatoxin become high in concentration i-e 22ng/g and 23ng/g in bottom sack hence if moisture level maintained during storage than wheat can be stored for long period by limiting the contamination, rest of the concentration of aflatoxin were recorded under the permissible limit set by European level.

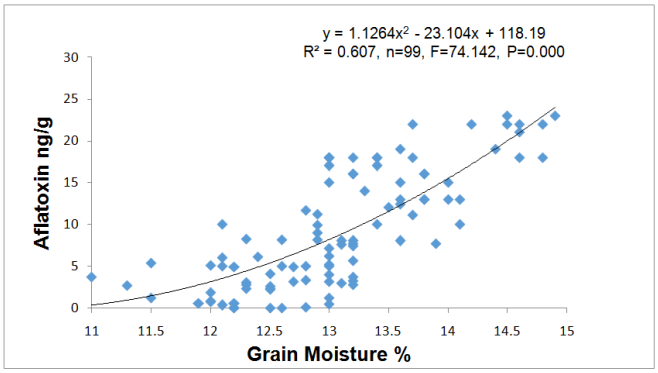
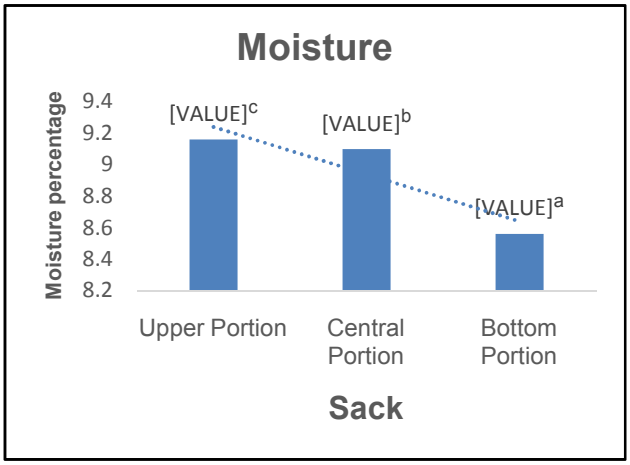


Figure 4. Regression curve of Aflatoxin Concentration (ng/g) in relation with grain Moisture Percentage

The 99% contamination was observed in summer wheat whereas 75% was in the sample of winter but the level of total aflatoxin was recorded within the permissible limit present data is in resemblance (Taheri *et al.,* 2012). Out of 83 wheat samples 1.2% of samples were positive for *AFB1* with concentration of 25.6 µg/Kg (Abdullah *et al.*, 1998). Highest contamination of aflatoxin were found in whole wheat grain whereas the overall average was 0.69 µg/kg which was under the limit of Brazilian legislation (5 µg/kg) only one sample exceeding this limit (Trombete *et al.,* 2014). Higher concentration of mycotoxin was found that is 15ppb in wheat flour (Mahmoudi *et al.*, 2012). In rice higher aflatoxin concentration were reported (Yazdanpanaha *et al.,* 2013). Among 156 peanut samples analyzed for AFB1, one sample (0.64%) at the level of 491 ng/g was contaminated (Khamiri *et al.,* 2008).

Data for the chemical analysis in whole wheat flour of godown stored wheat were recorded in Figure 5 the results revealed that moisture (%) of all contaminated wheat was in the narrow range of moisture 8.56-9.16% in all portioned wheat samples, the crude protein in bottom wheat showed higher percentage due to more contamination i-e 11.99% as compared to upper and middle crude protein were 10.33-10.99% respectively and same is for the crude fiber which increases with increase in the concentration of contaminated wheat samples. whereas the results showed that crude fiber percentage was bit decreased as contamination increases at the bottom sack wheat. The percentage of dry and wet gluten was found decreased due to higher contamination of aflatoxin producing fungi i-e in the wet gluten upper portion 26.91, central 25.22 and bottom sack showed 22.11% whereas upper, central and bottom portion wheat sack 10.68, 10.30, 9.67% respectively of dry gluten was observed. Lower quantity of wet and dry gluten in highly contaminated wheat samples was due to the damaged of gluten fractions by proteolytic enzymes.



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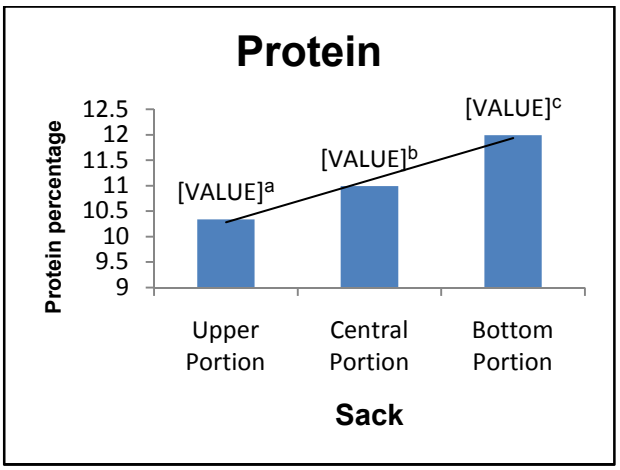

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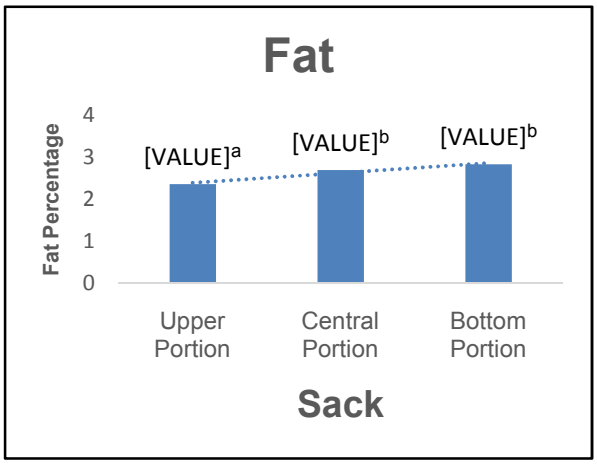

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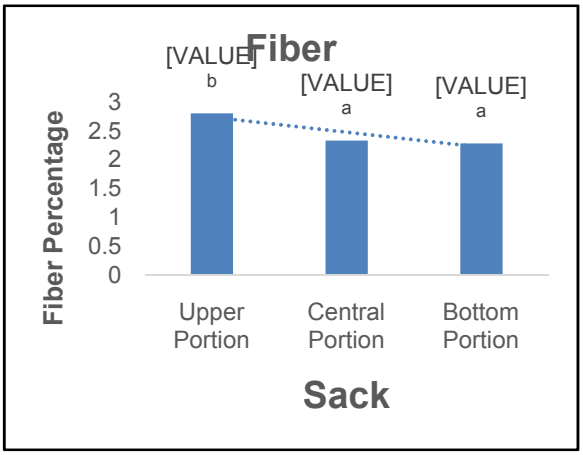

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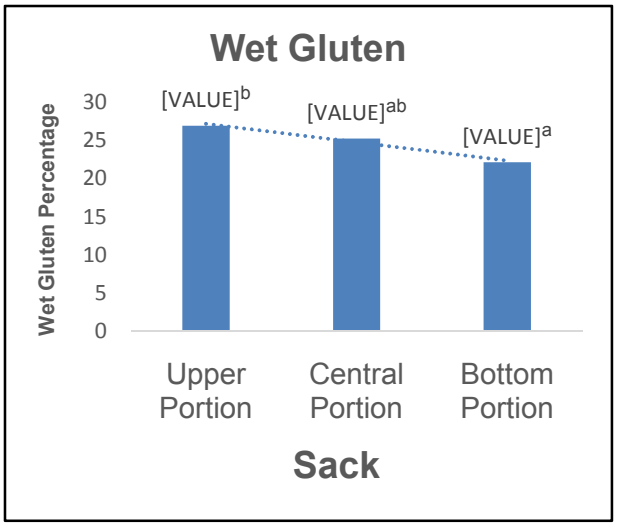
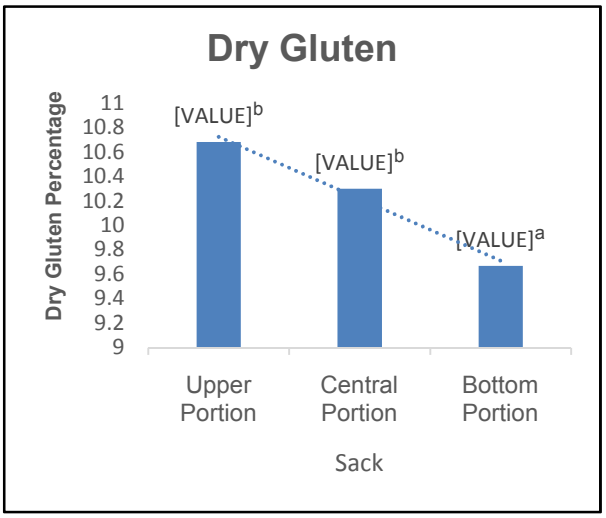


e)



f)

(a) Moisture % (F= 4.409, P=0.021, df = 32); (b) Crude Protein % (F= 22.45, P=0.00, df = 32); (c) Fat % (F= 9.880, P=0.001, df = 32); (d) Crude Fiber % (F= 9.87, P=0.0011, df = 32); (e) Wet Gluten % (F= 4.938, P=0.0210.14, df = 32); (f) Dry Gluten% (F= 6.323, P=0.14, df = 32).

Figure-5. Proximate analysis of highly contaminated wheat samples

(12.04%), higher fat content (3.0%), lower fiber content

(2.58%) and lower wet and dry gluten in samples contaminated with *A. Flavus* (Embaby *et al.,* 2012). *Aspergillus spp*. damaged the stored wheat and adversely affect on the nutritional composition (Chandra *et al.,* 2011). The results are also in agreement with previously reported data of Jackowiak, *et al.* (2005); Boyacioglu and Hettiarachchy (2005).

Conclusion

It was sum up from this study that aflatoxin producing fungi (*A. parasiticus and A. flavus*) increase their growth during storage period due to high moisture percentage of grain, under this favorable condition the frequency percentage becomes higher in the wheat of October month as compared to May and July whereas, the wheat samples taken from bottom portion of stack showed more frequency (%) among upper and central portioned stack due to high humidity, therefore in Dadu, Sehwan, K.N Shah and Aarazi after monsoon rainfall higher concentration of aflatoxin was noted. The storage condition can be improve by maintaining temperature and humidity level which provide the conditions unsuitable for growth of fungi and thus contamination ratio will be low and wheat can be stored maximally with the lowest contamination rate.

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