EFFECT OF MOISTURE CONTENT ON AFLATOXIN PRODUCTION IN FIELD INFECTED AND FARMER SAVED SORGHUM (FSS) GRAINS

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Abstract

Aflatoxin producing fungi and aflatoxins have been found to present and reported in several agricultural and food commodities led to the present study to be undertaken to determine and to find out the relationship between the moisture content (MC) of the Farmer Saved Sorghum grains (FSS) samples collected from the selected districts viz., Dharmapuri, Namakkal and Salem, Tamilnadu, India. Hence the samples of FSS, first estimated for its moisture content (MC) and then analysis for its Aflatoxin and multitoxin content by the method of Thin layer chromatography. Almost all the samples were with the limit of above 17% of moisture content. The toxigenic samples of 15 were reported for only aflatoxins B1 and 3 samples for aflatoxin B2 and none were reported for multitoxins other than AFB1 and AFB2. Aflatoxin B1 was produced only by the samples with the moisture content (MC) are, above the limit of 20%. These results revealed that there is strong correlation and positive relationship between MC to the production of AFB1 and AFB2 are ranges from only 5 ppb – 20 ppb which is considered to be at the safest proposed regulatory level, in India.

Keywords: Aflatoxin B1, B2, Moisture content, Thin layer Chromatography, FSS.

Introduction

Aflatoxin is a mycotoxin which are poisonous chemical compounds that are produced by certain fungi. Among the fungi, mycotoxins are produced both by field and storage fungi, that invade before harvest or can be occurred only after harvest respectively. According to several reports, the three toxigenic field fungi have been the Aspergillus flavus (aflatoxin), Fusarium (Fumonisins) and Penicillium (Ochratoxin) which are considered to be the predominant ones (Ayalew, 2010). The production of mycotoxins by certain fungi are due to the unfavourable conditions that are instigated by poor hygienic conditions especially during the storage time, high temperature and moisture content (Food Nutrition and Agriculture FAO, 1991). Milk is considered to be the main source of mycotoxins for the risks that can be caused in human health. (Gizachew, et al., 2016) and these secondary metabolites are also ubiquitous and easily accessible from different materials also. Aflatoxin fungi (Aspergillus flavus) is commonly distributed in the soil of temperate and tropical areas. (Gourami et al.,
1995). The strains of \textit{A. flavus} are likely to produce AFB1 mainly and with varying amounts of AFB2, AFG1 and AFG2.

Hence the present study is aimed to detect aflatoxin both qualitatively and quantitatively from the Farmer Saved Sorghum grains (FSS) as all cereal crops can contain aflatoxins. This is due to decreased genetic diversity and intensive cereal cropping practices contribute to preharvest increased infections in the agricultural commodities with fungi that produce aflatoxins. (Brown, et al., 1999; Lillehoj, 1992).

\textbf{Materials and Methods}

The shrivelled, discoloured, multicolored grains due to various colored, fungal mycelium which have been stored by farmers were collected from the study sites, the selected districts of central Tamilnadu, viz., Dharmpuri, Namakkal and Salem, India. All the 21 samples of collected FSS grains were estimated for its moisture contents by the Hot air oven method (AOAC, 2006) and estimation of multitoxin and aflatoxin was carried out by Romer’s method of Thin layer Chromatography (AOAC, 2006).

\textbf{Results and Discussion}

\textit{A. flavus} has led to extensive studies on the presence of mycotoxins in food stuffs since it has been discovered as aflatoxin is a potent mycotoxin produced by the fungi as reported by Borker (1966), Ciegler et al., (1968), Hesseltine et al., (1966) and Wogen (1965).

In the present study, quantitative estimation of aflatoxin from the 21 FSS samples collected from the study sites were assessed by the TLC method concorded by Pons and Goldblatt in 1969 itself, as they suggested that chemical methods for extraction, chromatographic methods for purification and methods for measurement of aflatoxin levels were developed, once the toxin hazard became the standard for analysis as well as screening of aflatoxins and is used simple and inexpensive, so used in the present study also. Official validation methods for analysis have been published by the AOAC (2006).

Squire (1981) reported that aflatoxin are difuranocoumarin derivatives. There are four major aflatoxins B1, B2, G1 and G2 with the letters referring to the colour of their fluorescence under ultraviolet light (blue or green) and the numbers indicating their relative migration distance on a thin-layer chromatographic plate. Cast (2003) and Gilbert and Vargas (2005) reported that there are other methods including HPLC (high-performance liquid chromatography, gas chromatography and immunological methods such as ELISA (Enzyme-linked immune-sorbent assay)) are also used.

Van Egmon and Vonker (2005) (http://www.fao.org/do007/y5499e/Y5499e07.htm;) reported that regulation levels for aflatoxins vary from country to country which are at least in
place in 99 countries. According to this regulatory levels are set at 20 parts per billion (p.p.b., \( \mu g/kg \)) for human food, 0.5 p.p.b. for milk and up to 300 p.p.b for corn and cotton seed in animal feed. However, in India for all foods regulatory levels are set to 30 p.p.b.

According to Yazdani et al (2010), although many techniques have been increasingly applied in the determination of certain varieties of mycotoxins, the use of culture media for detection did not appear advisable. HPLC and TLC involving testing for actual extracts of the toxins were confirmed to be the only reliable methods for toxin detection although other tests could be used as preliminary indicators.

The finding shows that the moisture content has been was highest in the FSS grains. Most of the investigators found that there is a strong relationship between moisture and the grain deterioration in stored conditions. Because according to them, despite the fact that grain may be uniform and within what is normally considered a safe moisture limit at the outset of storage, fungal deterioration may still result because of excessive moisture. According to Christensen (1972) and Milner and Geddes (1954), equilibrium moisture content for sorghum ranges near 15.3%. Lillehoj et al., (1975A, 1977) Caldwell and Tuite (1974) have also reported that *Aspergillus flavus* can invade in the field and can continue to decay grain in storage along with *Fusarium* if the moisture is high enough.

Out of the 21 samples collected from the study sites, 15 were found to be positive ranging from 5 ppb to 20 ppb of AFB\(_1\) and 3 samples with 5 ppb of AFB\(_2\) and none were detected for other toxins. The same was disclosed by Abraham and petros (1981) who have detected 5 ppb of aflatoxins in some selected Ethiopian foodstuffs maize, sorghum and teff injera samples. The same was also made by Dawit (1982) a qualitative study of aflatoxins on maize, sorghum, teff and barley with a special emphasis on toxicogenic fungal groups, assumed that maize and sorghum are more often associated with aflatoxicosis than teff and barley.

However sufficient information is not available on the quantitative level of aflatoxins of poorly stored cereals especially sorghum grains, the most commonly consumed food product of India. No work has also been conducted on the effects of certain environmental factors that are known to effect the formation of Aflatoxins in cereals was the observation made by Goreleva (1980) and Jarvis (1971). Hence the present study comprise the determination of the Aflatoxins contents of commonly used cereals jowar and the effects of moisture in collected FSS grains.

Table results shows AFB1, with 71.42% and AFB2 with 14.28% of the total 15 samples ranging from 5 ppb to 20 ppb and 5 ppb irrespective of FSS grains collected in the
study sites. However, the results of this study in general, seems to support the conclusion reached by Abraham and petros (1981) because only small proportion, 71 (14.0%) of the examined samples were positive for aflatoxin and the larger proportion (91.5%) of the positive samples had aflatoxins below the recommended upper tolerance limit of 30 ppb, which, later content was reported by WHO (1983) and Christensen (1972).

**ESTIMATION OF MYCOTOXINS FROM FARMER SAVED SORGHUM GRAINS**

<table>
<thead>
<tr>
<th>LOCALITY</th>
<th>SAMPLE NO.</th>
<th>SAMPLE TYPES</th>
<th>MC%</th>
<th>MOISTURE CONTENT</th>
<th>AFB1 ppb</th>
<th>AFB2 ppb</th>
<th>MULTITOXIN IN OTHER THAN AFB1 &amp; AFB2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>DHARMAPURI</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
<td>Field Infected Grains</td>
<td>17.03</td>
<td>ND*</td>
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<td></td>
</tr>
<tr>
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<td></td>
<td>Field Infected Grains</td>
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<td>-</td>
<td></td>
</tr>
<tr>
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<td></td>
<td>Field Infected Grains</td>
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<td>ND*</td>
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<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td>Field Infected Grains</td>
<td>22.79</td>
<td>15</td>
<td>ND*</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td></td>
<td>Farmer Saved Grains</td>
<td>25.34</td>
<td>15</td>
<td>-</td>
<td>-</td>
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</tr>
<tr>
<td>6</td>
<td></td>
<td>Farmer Saved Grains</td>
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<td>15</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Farmer Saved Grains</td>
<td>28.35</td>
<td>10</td>
<td>5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><strong>NAMAKKAL</strong></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
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<tr>
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<td>ND*</td>
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<td>ND*</td>
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<td></td>
<td>Field Infected Grains</td>
<td>20.27</td>
<td>5</td>
<td>ND*</td>
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<tr>
<td>4</td>
<td></td>
<td>Field Infected Grains</td>
<td>20.88</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
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<td>5</td>
<td></td>
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<td>24.79</td>
<td>10</td>
<td>5</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
<td>Farmer Saved Grains</td>
<td>28.20</td>
<td>10</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td></td>
<td>Farmer Saved Grains</td>
<td>31.36</td>
<td>20</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

**Moisture (x) Vs Aflatoxin (y)**

- **DHARMAPURI**:
  \[ y = 1.096x - 16.683 \]
  \[ R^2 = 0.4202 \text{ (Positive)} \]

- **NAMAKKAL**:
  \[ y = 1.1803x - 19.126 \]
  \[ R^2 = 0.8458 \text{ (High, Good, Positive)} \]
Table also indicates the overall results of Aflatoxins in all the collected grains samples. Out of the 21 samples AFB1 ranging from 5 ppb to 20 ppb were detected from 10 samples of FSS grain and 5 samples for freshly infected seeds with the percentage of 66.7% and 33.3% respectively. Abovementioned results seem to agree with the work of other investigators present result also indicates the occurrence of Aflatoxins content was higher in the FSS grains with the high moisture percentage. Moreover, aflatoxins were detected properly in the grains with the high moisture contents. Hence the study reveals that AFB1 and AFB2 was detected in the grains where the moisture content is ranging from 20 – 30%. These results were correlated with the observation that 15.5 – 20% moisture content as optimum ranges for aflatoxin formation and 22 – 35% moisture content as the maximum limits and 13 – 13.5% moisture contents as the minimum limits made by Dawit (1982), Jarvis (1971), Schoredar (1967) and Christensen (1972).

Hence above results have a conclusion that though it is true that 30 ppb is the upper acceptable limit of aflatoxins for some foods, some workers have suggested that 15 – 30 ppb level of aflatoxins could be considered dangerous to the health recorded by Goreleva (1980), WHO (1983) and Boltyanskaya (1980). Since in this study it has been shown most sorghum samples seems that they are at little risk, in view of the results, some preventive and detoxifications measures of aflatoxins from cereals, care can be taken during the cultivation, harvesting and storage periods of all grain seeds.

Based on the results of current study, AFB1 production was increased proportionally with moisture content. Hence in stored condition, grains should be kept at low moisture

<table>
<thead>
<tr>
<th></th>
<th>Field Infected Grains</th>
<th>17.72</th>
<th>ND*</th>
<th>-</th>
<th>-</th>
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<tbody>
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<td>2</td>
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<td>ND*</td>
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<td>-</td>
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<td>3</td>
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<td>3</td>
<td>ND*</td>
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<td>4</td>
<td>Farmer Saved Grains</td>
<td>22.78</td>
<td>10</td>
<td>-</td>
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</tr>
<tr>
<td>5</td>
<td>Farmer Saved Grains</td>
<td>23.00</td>
<td>15</td>
<td>-</td>
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</tr>
<tr>
<td>6</td>
<td>Farmer Saved Grains</td>
<td>23.35</td>
<td>15</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Farmer Saved Grains</td>
<td>23.64</td>
<td>10</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* ND = Not Detected

\[ y = 2.2662x - 40.385 \]
\[ R^2 = 0.8728 \]
(High, Good, Positive)
Chamarthy venkatarathanavathi et al., (2011) worked on natural occurrence of AFB$_1$, in sorghum grown in different geographical regions of India revealed that the grains showed a significant difference for AFB1, contamination year to year. This was mainly due to the variation in fungal colonization, especially A. flavus and is highly influenced by the weather condition prevailing during the grain development stage. Hence, he suggested the contamination of mycotoxins in sorghum is low to medium in Kharif produce and grain is mostly safe for consumption, and awareness campaigns among farmers and poultry feed manufacturers and consumers should be in Kharif-sorghum-growing areas.

Detection of AFB$_1$ was the most occurring in sorghum in their products was also recorded as that our findings by Apeh Daniel ojachenemi et al., (2016). Contamination of sorghum grain (71.5%) by aflatoxin is similar to Makun et al., (2009) who reported 31.25% and 57.85% in field and stored sorghum samples. It is similar to the results obtained in the study, was 33.3% and 66.7% respectively. But Uriah and Ogbadu (1980) found 100% contamination of sorghum samples in Northern Nigeria while oxpadoxun found 6.9% contamination.

Almost 71% of the samples of three zones were recorded with AFB1 contaminant uniformly. Of the hundreds of the mycotoxins occurring in nature, aflatoxin is one among the five agriculturally important fungal mycotoxins as reported by Bhat et al., (2003) which were found in abundance in foods especially grains including sorghum and pose a vast array of scientific problems and challenges to food production, public health and international trade. Several investigations including this study have detected AFB1 from sorghum in the study sites and other parts of the globe was reported by Okoye (1992) and Uraguchi (1978).

**Conclusion**

Scarcity of food, disasters and poverty leave many people in this region of the world with little or no option but to purchase and consume low grade cereals. The consumption of mouldy grains by man and animals has severe consequences on public health, particularly that the mycotoxins are found at unsafe levels in the mouldy grains (Makun Hussaini et al., 2009). This study was a biased one because only mouldy samples of sorghum were sampled for the work. Though such studies cannot give useful incidence data like unbiased study, biased analysis can give natural fungal and mycotoxin profile of an area. Such biased studies are also becoming increasingly important in public health hazards in India, because cheaper low grade are fed to animals.
References


