Abstract—A survey was conducted in the five rice-growing regions in Guyana to determine the presence of aflatoxins in multiple fractions of rice in June/October 2015 growing season. The fractions were paddy, steamed paddy, cargo rice, white rice and parboiled rice. Samples were analyzed by High Performance Liquid Chromatography. A subset of the samples was further analyzed by enzyme-linked immunosorbent assay (ELISA) for concurrence. All analyses were conducted at the University of Missouri, USA. Of the 186 samples tested, 16 had aflatoxin concentrations greater than 20 ppb the recommended limit for aflatoxins in food according to the United States Food and Drug Administration. An additional three samples had aflatoxin B1 concentrations greater than the European Union Commission maximum levels for aflatoxin B1 in rice at 5 µg/kg and total aflatoxins (B1, B2, G1 and G2) at 10 µg/kg. The survey indicates that there is no widespread aflatoxin problem in rice in Guyana. The incidence of aflatoxins appears to be localized.

Keywords—Aflatoxins, enzyme-linked immunosorbent assay, high-performance liquid chromatography, rice fractions

I. INTRODUCTION

Rice is a very important commodity in Guyana. It is our staple. The rice industry is the second most important agricultural industry and the largest use of agricultural land in Guyana. The rice industry is also a major source of income and employment. Rice is grown in six of Guyana’s ten administrative Regions, Region 2, 3, 4, 5, 6, and 9, with Region’s 9 production being negligible (Table I, Fig. 1). Guyana, exported 0.5 million tonnes of rice in 2015 [1]. The country’s annual rice production is continuously expanding along with its international rice market share, with exports to most continents (including Europe and Asia) and to CARICOM countries [1], [2]. The quality of Guyana’s rice yield, its export markets, its international market share and reputation can potentially be diminished by the presence of harmful microorganisms and their metabolites in its rice products.

One of the most important pathogens that can impact Guyana’s rice industry is the Aspergillus fungus. There are three sections and thirteen species of the genus - Flavi, Nidulantes and Ochraceorosei- that produce the secondary metabolite aflatoxin [3]. *Aspergillus flavus* belongs to the section flav. This section contains the major economically important aflatoxin-producing species *A. flavus* and *A. parasiticus* [4].

<table>
<thead>
<tr>
<th>Region</th>
<th>Hectar Harvested</th>
<th>Paddy Production</th>
<th>Rice Equivalent</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Bags</td>
<td>M/T</td>
<td>M/T</td>
</tr>
<tr>
<td>Region 2</td>
<td>29,984.30</td>
<td>2,502,975</td>
<td>158,991</td>
</tr>
<tr>
<td>Region 3</td>
<td>17,555.20</td>
<td>1,386,107</td>
<td>88,047</td>
</tr>
<tr>
<td>Region 4</td>
<td>6,785.00</td>
<td>696,663</td>
<td>44,253</td>
</tr>
<tr>
<td>Region 5</td>
<td>80,153.20</td>
<td>6,415,866</td>
<td>275,597</td>
</tr>
<tr>
<td>Region 6</td>
<td>50,139.20</td>
<td>4,338,684</td>
<td>275,597</td>
</tr>
<tr>
<td>Region 9</td>
<td>405</td>
<td>45,024</td>
<td>2,860</td>
</tr>
<tr>
<td>Total</td>
<td>185,021.75</td>
<td>15,385,319</td>
<td>977,289</td>
</tr>
</tbody>
</table>

Fig. 1 Map of Guyana showing the rice growing Regions (Regions 2, 3, 4, 5, 6 and 9) [6]

There are four major aflatoxins, B1, B2, G1 and G2 with B1 usually being the aflatoxin in highest concentration in contaminated feed and food including rice [7], [8]. Aflatoxin B1 is also the most potent and is rated as a Group 1 carcinogen because there is evidence that it causes hepatocellular carcinoma in humans [9]. In 2004, 125 Kenyans died of acute...
aflatoxicosis: a disease associated with consuming extremely high levels of aflatoxin in food [10]. The symptoms of aflatoxicosis include hemorrhagic necrosis of the liver, bile duct proliferation, edema and lethargy. In 2006, Brazil had an outbreak of the disease beriberi associated with poor rice storage resulting in at least 47 deaths and it was suggested that this may have been due to aflatoxins [11]. It was also determined that there is an increased risk of lung cancer for persons exposed to dusty conditions in rice mills [12]. Cytotoxicity and apoptosis occur in the lungs as a result of affected respiratory reactive centers [13]. With such potentially harmful effects on health, consideration should be given to the variety of rice cultivated, and pre-harvest conditions. For example, it was determined that the variety super basmati had the highest aflatoxin concentration among four varieties of rice that were studied [14]. While [15] determined that aflatoxin-producing A. flavus inoculated Basmati rice produced more aflatoxins than aflatoxin-producing A. flavus inoculated normal rice. As the plant develops, environmental conditions can influence the presence of the fungus and its susceptibility to fungal infection. In Nigeria, it was observed that in the dry harnamattan Aspergillus niger was the predominant fungal species present in the soil while in the rainy season it was Aspergillus flavus.

Aflatoxin contamination had temporal and spatial variation with contamination being directly correlated with rainfall [16]. Soil surface temperature also greatly influences fungal communities and they thrive best in a temperature range of 18 to 30 °C, the fungal propagule density decreases outside this range [17].

Postharvest conditions during storage are also critical in the monitoring and controlling of the fungus. Reference [18] posits that postharvest loss in developing countries is between 30 to 50% in value. In India, paddy collected from the field had significant levels of Aspergillus spp. with Aspergillus flavus dominating followed by Aspergillus niger producing AFB1 [19]. Hence a range of aflatoxins was found in freshly harvested paddy [20]. Growth of the fungus and subsequent production of toxins take place in the harvested paddy where the water activity (a_w) was in the range 0.86-0.99 with the optimal a_w at 0.98, and an optimal temperature in the range of 25-30 °C [21]. Due to Aspergillus’ ability to grow at low water activity when storage conditions are poor, paddy becomes contaminated with aflatoxins. Rice inoculated with A. flavus, milled and stored at 21 °C and 95% relative humidity and 30°C/85% RH produced AFB1 and AFB2 during the 120 day storage period with brown rice having more aflatoxins than rough and white rice [22]. In the Philippines, rice and rice by-products had high levels of Aspergillus flavus and aflatoxins which pose a risk to health due to poor post-harvest handling and storage [23]. On the other hand, in Japan, moisture content and temperature are strictly regulated in warehouses. As a result, there were no detectable levels of aflatoxin in rice [24].

Milling during and after storage to different rice fractions are also potential sources of contamination. Aflatoxin B1 was the highest Aspergillus toxin produced in milled rice [25]. Investigations on different fractions of naturally contaminated rice revealed that brown rice had the highest percentage of aflatoxins followed by bran and white rice [26]. This result concurs with [27] where it was observed that paddy had the highest concentration of aflatoxins, followed by parboiled rice, brown rice and white rice with broken rice having the least. It was suggested that the process of preparing parboiled rice which involves precooking and soaking followed by drying and then milling might predispose it to aflatoxin contamination. Hence, [28] noted increased levels of aflatoxins following the drying and milling processes. Aflatoxins above the regulatory levels were observed in parboiled rice in India and Iran [29]-[31].

Given the severity of aflatoxin toxicity, some countries are endeavoring to determine the extent of their population’s exposure to aflatoxins. For example, in Shenzhen (China), it was determined that that the 7-14 age group was more vulnerable to aflatoxin exposure from the main dietary ingredients rice and peanut oil [32]. In Kenya, the population is at high risk for aflatoxicosis from their staple diet of maize [33]. Lebanese are also exposed to high levels of aflatoxins in their diet [34]. In contrast, [35] reported that dietary intake of aflatoxin B1 in Japan did not pose any health risk. The United States Food and Drug Administration (USFDA) set the limit of aflatoxins in food to 20 µg/kg while the European Union (EU) Commission has set the maximum levels for aflatoxin B1 in rice at 5 µg/kg and total aflatoxins (B1, B2, G1 and G2) at 10 µg/kg since rice is subjected to physical treatment before human consumption [36], [37].

This study seeks to provide information for Guyana’s rice industry on the quality of paddy and milled fractions of rice. Specifically, it seeks to determine (i) the total aflatoxins (AFB1, AFB2, AFG1 and AFG2) in paddy, cargo rice, white rice, and parboiled rice in five rice-growing regions in Guyana and (ii) if there are differences in aflatoxin concentration among the regions.

II. MATERIALS AND METHODS

A. Sample Collection and Preparation

One hundred and thirty five (135) samples of paddy, white rice, cargo rice and parboiled rice were collected from 50 rice mills along with 51 samples of paddy produced by farmers from the five main rice-growing regions in Guyana, viz. Regions 2, 3, 4, 5, and 6 (Fig. 1). The samples were collected in the June/October 2015 growing season, which corresponded to the rainy season. The samples were taken to the laboratory of the University of Guyana where the paddy and rice were ground with a Stein M-2 sample mill (15,000RPM) for one minute. All samples were sealed in appropriately sterilized containers then transferred for analysis to the Veterinary Medical Diagnostic Laboratory located at the University of Missouri, USA.

B. High-Performance Liquid Chromatography - Aflatoxin Extraction and Purification

The aflatoxins were extracted by adding 100 mls of acetonitrile: water solution (7:3) to 25 g of the ground rice and
Paddy collected from farmers had the highest mean aflatoxin concentration followed by parboiled rice. The analysis of variance indicates that there are no significant differences among the different rice fractions.

The values obtained were due to detectable presence of aflatoxins in all fractions except cargo rice. The paddy collected from the farmers had the widest range of aflatoxin concentration and the highest concentration of total aflatoxin. The concentration of aflatoxin B1, which is the most toxic of the four aflatoxins, made up 17 to 90 % of all aflatoxins with an average of 48% of the total aflatoxin concentration for the entire sample set. Paddy had a higher aflatoxin concentration than white rice and parboiled rice findings consistent with observations by [27]. However, parboiled rice had the highest percentage of samples above the European standard of 10 µg/kg (Table III). These results for parboiled rice are very similar to [38] where aflatoxin concentration in the range 11 to 74 µg/kg was observed in 9% of the tested parboiled samples.

The method used for the preparation of parboiled rice may allow for the growth of fungus since in some cases the paddy is soaked in tanks for between 48 to 72 hours, drained, steamed and then dried before milling. There is a correlation between the soaking period and aflatoxin concentration [31] while [38] noted that the duration of soaking influences the
migration of aflatoxin from the husk to the endosperm. Of the nineteen samples that tested positive, just over half of the samples (52.6%) were gathered from Region 6. This is a reflection of the Region’s focus on producing parboiled rice which had the highest percentage of aflatoxin contamination (Fig. 2). Region 3 had no positive samples. In spite of this observation, analysis of variance showed that differences in aflatoxin concentrations were not as a result of variation among the Regions. Hence, geographical location did not influence the presence of mycotoxins, a finding in contrast to results obtained by [39] where geography and climate contributed to the differences observed among six provinces in China. The greater size and differences in environments across the much larger provinces in China compared to the much smaller regions in Guyana may have contributed this difference in results.

<table>
<thead>
<tr>
<th>TABLE III</th>
<th>OCCURRENCE OF AFLATOXINS IN SIX FRACTIONS OF RICE PRODUCED IN GUYANA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample fraction</td>
<td>Number of samples analysed</td>
</tr>
<tr>
<td>Farmers' paddy</td>
<td>50</td>
</tr>
<tr>
<td>Paddy from mills</td>
<td>38</td>
</tr>
<tr>
<td>Steamed paddy</td>
<td>19</td>
</tr>
<tr>
<td>White rice</td>
<td>35</td>
</tr>
<tr>
<td>Cargo rice</td>
<td>12</td>
</tr>
<tr>
<td>Parboiled rice</td>
<td>30</td>
</tr>
<tr>
<td>TOTAL</td>
<td>184</td>
</tr>
</tbody>
</table>

The Rida® Quick aflatoxin RQS ECO method has a detection range of 4-75 µg/kg. Therefore, only those samples within that range from the results of the analysis by HPLC were evaluated by ELISA. The ELISA results indicated that samples that tested positive with HPLC were also positive using the ELISA. Concurrence between ELISA and HPLC was also obtained in quantifying aflatoxins in brown rice [40]. In outlining the advantages of both methods, [41] indicates that the sensitivity and specificity of HPLC are higher than the ELISA method.

The small percentage of samples with aflatoxin may be indicative of the persistent efforts by the Quality Control Department of the Guyana Rice Development Board to ensure the production of good quality rice for local consumption and international export. In Guyana, the head office of the Guyana Rice Development Board is located in the city. However, in each rice-growing Region, there is an office with qualified personnel to discharge the mandate of the Board by interfacing with farmers and millers in the Region. The Board, however, should examine areas of weakness such as the process for making parboiled rice and make recommendations for improvement. It should also focus on monitoring Regions such as 4/5 for sanitary practices in the field and factory in an effort to minimize aflatoxin contamination. In addition, the rice industry may endeavor to become like Japan applying best practices in the warehouses where the humidity, temperature and moisture content are controlled, thus ensuring that the rice is free of aflatoxins [24].

IV. CONCLUSION

The survey indicates that there is no widespread aflatoxin problem in rice in Guyana. Incidence of aflatoxins appears to be localized. However, it is important to continue monitoring so that the status quo can remain, while endeavoring to improve by encouraging more millers to upgrade their facilities to current best practices. This will assist in expanding Guyana’s rice market share and ensuring the health and safety of consumers.

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REFERENCES


