

The Determination of Aflatoxins in Paddy and Milled Fractions of Rice in Guyana: Preliminary Results

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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 flavi. This section contains the major economically important aflatoxin-producing species *A. flavus* and *A. parasiticus* [4].

TABLE I
GUYANA'S RICE PRODUCTION IN 2014 [5]

Region	Hectare Harvested	Paddy Production		Rice Equivalent
		Bags	M/T	M/T
Region 2	29,984.30	2,502,975	158,991	103,344
Region 3	17,555.20	1,386,107	88,047	57,230
Region 4	6,785.00	696,663	44,253	28,764
Region 5	80,153.20	6,415,866	407,541	264,902
Region 6	50,139.20	4,338,684	275,597	179,138
Region 9	405	45,024	2,860	1,859
Total	185,021.75	15,385,319	977,289	635,238

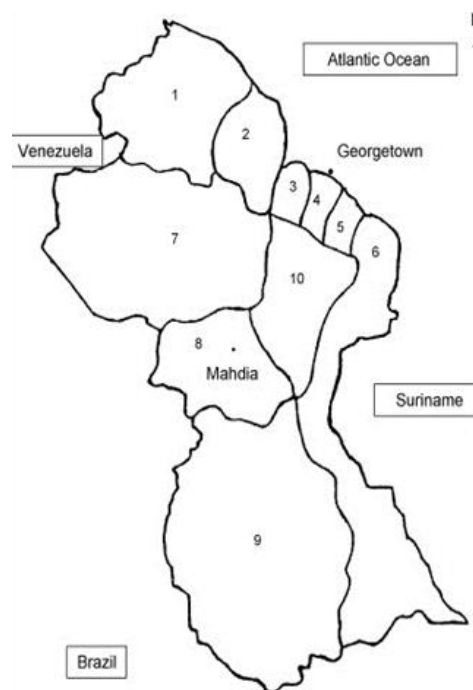


Fig. 1 Map of Guyana showing the rice growing Regions (Regions 2, 3, 4, 5, 6 and 9) [6]

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

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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 harmattan *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 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

ground paddy. This mixture was placed on a rotary shaker for 30 minutes. The supernatant was cleaned and filtered using PuriToxSR aflatoxin cleanup columns (TC-M160) purchased from Trilogy Analytical laboratory.

C. High-performance Liquid Chromatography - Analysis of Extracts

The rice sample extracts were analysed for total aflatoxins consisting of AFB₁, AFB₂, AFG₁ and AFG₂ by HPLC. These were individually determined after derivatization with bromine using a KOBRA cell. The mobile phase was methanol:acetonitrile:water (1:1:4) with the addition of 1.2 g potassium bromide and 360 µl of nitric acid. The chromatographic parameters were: flow rate of 0.9 ml/min, injection volume of 50 µl with the column at room temperature. Aflatoxins were detected using a scanning fluorescence detector ($\lambda_{\text{ex.}} = 360 \text{ nm}$, $\lambda_{\text{em.}} = 440 \text{ nm}$). These were quantified using retention time, peak area and external calibration curves. The approximate retention times for AFB₁, AFB₂, AFG₁, and AFG₂ were 14.2 minutes, 10.4 minutes, 9.5 minutes and 7 minutes respectively. The linearity of the HPLC method was determined using correlation coefficients of the calibration curves of known concentration of standards.

D. Analysis by ELISA

Samples were analyzed using the RIDA®QUICK aflatoxin method. The aflatoxins were extracted by adding 20 mls of methanol:water solution (7:3) to 10 g of the ground feed samples and mixed by vortexing for 10 seconds. This mixture was placed on a rotary shaker for 5 mins, then centrifuged for 3 minutes. Fifty (50) µl of the supernatant was placed in an Eppendorf microtube, 100 µl of the mobile solvent provided with the RIDA®QUICK kit was added and mixed by placing on a vortex for ten seconds. Of this solution, 100 µl was placed on the test strip insert and allowed to incubate for 5

minutes. The insert was then placed in the RIDA®QUICK measuring device and scanned.

E. Statistical Analysis

The samples were assessed based on USFDA limit of aflatoxins in food of 20 µg/kg and EUC maximum levels for aflatoxin B₁ in rice of 5 µg/kg and total aflatoxins, that is B₁, B₂, G₁ and G₂ of 10 µg/kg [36], [37]. For statistical analysis, the mean, minimum, and maximum values were estimated. Analysis of variance ($p < 0.05$) was performed for the fractions and regions to determine if they were statistically different. Since Region's 4 production and consequently sample collection was relatively small compared to the other Regions, it was analysed with Region 5 and represented as 4/5.

III. RESULTS AND DISCUSSION

Nineteen of the 186 samples had values higher than the European Union Commission standard of 10 µg/kg while sixteen of those samples were above the US Food and Drug Administration regulatory limit of 20 µg/kg. Two of the samples were considered as outliers. One sample was paddy collected from a farmer and the other was paddy collected from a mill with total aflatoxin concentrations of 13,984 µg/kg and 1,847 µg/kg, respectively. Both samples were from Region 4/5. These high aflatoxin concentrations may represent isolated incidences. However, given the critical food safety issue, and the importance of rice to Guyana's economy, the factors that might contribute to these high aflatoxin concentrations such as poor postharvest storage and handling must be investigated. Paddy, especially fresh paddy is susceptible to fungal contamination if not properly stored [20]. For each of the six fractions examined, the mean aflatoxin concentration of each of the fractions was below the European standard of 10 µg/kg total aflatoxins (Table II).

TABLE II
ANALYTIC RESULTS OF AFLATOXIN CONCENTRATION IN SIX RICE FRACTION.

Fraction	Number of samples analysed	Mean (µg/kg)	Standard deviation (µg/kg)	Minimum	Maximum
Paddy from mill	38	5.26	21.78	nd*	128
Steamed paddy	19	6.16	26.84	nd	117
Farmers' paddy	50	7.48	30.05	nd	193
White rice	35	1.11	4.78	nd	25
Cargo rice	12	0.00	0.00	nd	nd
Parboiled rice	30	6.77	17.65	nd	76

*Less than 10 µg/kg

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 III
OCCURRENCE OF AFLATOXINS IN SIX FRACTIONS OF RICE PRODUCED IN GUYANA

Sample fraction	Number of samples analysed	Number of samples with aflatoxin	% of sample fraction	Range of total aflatoxin concentration for positive samples ($\mu\text{g/kg}$)
Farmers' paddy	50	5	10	19 - 193
Paddy from mills	38	4	10.5	10 - 128
Steamed paddy	19	1	5.3	152
White rice	35	2	5.7	14 and 25
Cargo rice	12	0	0.0	0
Parboiled rice	30	5	16.7	11 to 41
TOTAL	184	17		

The Rida®Quick aflatoxin RQS ECO method has a detection range of 4-75 $\mu\text{g/kg}$. 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.

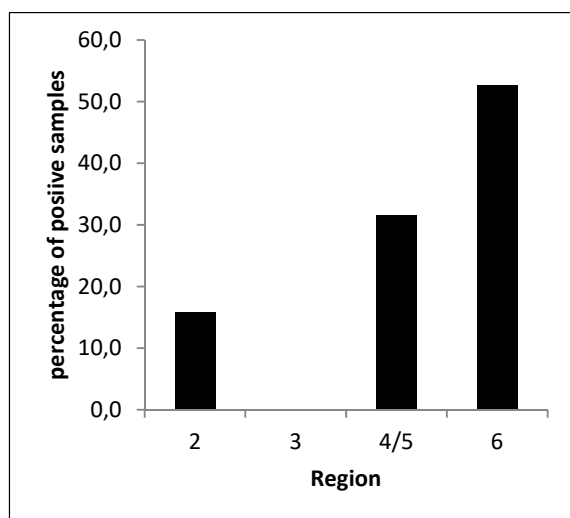


Fig. 2 The percentage of positive samples in the five regions

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.

ACKNOWLEDGMENT

The authors wish to thank the University of Guyana Science and Technology Support Project (UGSTSP) for the grant to conduct this project. We are also grateful to The University of Missouri (UM), Animal Sciences Division for its financial support, and Dr. George Rottinghaus and Mr. Ben Landers of UM Veterinary Medical Diagnostic Laboratory for their invaluable technical assistance.

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