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Status of aflatoxin B_1 in rice and rice products from Jhapa district of Nepal

Dev Raj Acharya[1], Lekhraj Dhakal[2], Santosh Thapa[3]*

[1]*Central Department of Food Technology, Institute of Science and Technology, Tribhuvan University, Nepal.*
[2]*Department of Food Technology, Sunsari Technical College, Dharan, Nepal.*
[3]*Department of Food Technology, Central Campus of Technology, Tribhuvan University, Dharan, Nepal.*

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ABSTRACT

The occurrence of aflatoxin in staple food products is a serious threat to public health. This studu aimed to determine the level of aflatoxin B1 (AFB1) contamination in rice and rice products produced in Jhapa, a major rice producing area of the country. A total of 108 samples including paddy, rice and rice-products (4 varieties each) were collected and the amount of AFB1 in them was analyzed using Bio-Shield B1 5 Enzyme-Linked Immunosorbent Assay (ELISA) test. The major varieties of paddy cultivated were Ranjit (26.61%), Sarana (22.22%), NR-2167 (13.89%) and Sukkha variety (5.56%). Fungal attack, color change and unwanted odor were major problems incurred during paddy storage while fungal attack and appearance of lumps were major problems during rice storage. About 76.92% of respondents were unaware of good agricultural practices and 87% of them had no idea about aflatoxins. Through ELISA, it was found that paddy, rice and rice products had a mean AFB1 content of 1.43, 1.41 and 1.64 µg/kg respectively, and the contamination levels differed significantly among different varieties of the samples. Ranjit variety of paddy, rice as well as beaten rice had the highest level of contamination among paddy, rice and rice product samples respectively. All the samples had AFB1 concentrations below the standards set by Nepal Government as well as World Health Organization. But 1 sample of Sukkha paddy, 3 samples of Ranjit paddy, 1 sample of Mansoori rice, 3 samples of Ranjit rice, 3 samples of Ranjit beaten rice and 3 samples of Mansoori puffed rice had AFB1 above the European Union standard.

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1. Introduction

Mycotoxins are secondary metabolites produced by micro fungi that are capable of causing disease and death in humans and other animals (1–3). They are one of the serious natural contaminants of many important plant products such as cereals, nuts, spices, dried fruits, etc. and they impose serious health problems for humans as well as animals (4–8).

*Corresponding author. Tel.: +9779814137214
E-mail address: thapasantosh919@gmail.com

The main fungi producing mycotoxins belong to the genera *Aspergillus*, *Fusarium* and *Penicillium* (2,9–11). The most common mycotoxins are aflatoxins, fumonisins, ochratoxins, cyclopiazonic acid, trichothecenes, ergot alkaloids, patulin and zearalenone (3,7,12–14). Aflatoxins are said to be the most important mycotoxins involved in food contamination worldwide, especially in the developing nations (15). They are highly toxic, mutagenic, teratogenic and carcinogenic compounds that have been implicated as causative agents in human hepatic and extrahepatic carcinogenesis (16–18). Among the aflatoxins, AFB1 is the most commonly occurring one as well as the most potent hepato-carcinogen known to man which has both acute as well as chronic toxicity (19).

Rice is one of the most consumed grains after wheat and is the staple food crop of more than a half of the world population (20,21). It is generally cultivated in subtropical environments with hot and humid climates (22). Such factors such as high temperature, high humidity, heavy rainfall followed by drought all increase the chance of aflatoxin contamination in rice (23). Similarly, during harvesting and storage, humid and warm climates associated with frequent rainfall increase the chance of aflatoxin production by fungal growth in rice (23,24). Several studies have reported the occurrence of aflatoxins in rice with a high prevalence in Asian countries (25–34). To ensure safety among consumers, regulatory bodies of different countries have set maximum permitted limits for AFB1 in rice. The European Union has set the maximum tolerable levels for AFB1 in rice at 2 μg/kg (Commission Regulation No. 1881/2006) (35) while the WHO has set the maximum levels at 30 μg/kg in food (36).

Nepal is an agricultural country where agriculture contributes 31.7% of total GDP among which 16.33% of agricultural GDP is contributed by paddy cultivation (37). According to Ministry of Agriculture and Livestock Development of Nepal, 5.61 million metric tons paddy was produced in Nepal during the fiscal year 2018/19 while 365,845 metric tons paddy was produced in Jhapa (38).

Occurrence of aflatoxins has been a serious problem in cereals produced in Nepal too (39–43). Nepal Government has also allocated the maximum permitted levels of aflatoxin in cereals and grain based foods to be 20 μg/kg (43). Although, some studies have been made to determine the level of aflatoxins in food commodities of Nepal, no study have been found to be performed regarding analysis of aflatoxin in the most consumed staple grain of the nation, i.e. rice. So, this study aims to study the status of AFB1 in paddy and paddy products in Jhapa, which is a major producer of rice of Nepal.

2. Materials and Methods

2.1. Study Area

Jhapa district is one of the largest producer of paddy in Nepal and is situated in eastern Terai. Thus, the rice super zone area of Baniyani (26°26′24″ N, 88°3′0″ E) was selected for the assessment of AFB1 concentration in paddy and paddy products in this study.

2.2. Survey

A survey was conducted by taking 25 respondents. They were asked about main variety of paddy cultivated, major problems incurred during paddy storage, major problems incurred during rice storage, idea regarding pesticide usage in farmers, knowledge regarding good manufacturing practices and knowledge about aflatoxins.

2.3. Sample collection

Samples of paddy, rice and rice products were collected from different areas within Baniyani, Jhapa. The variety of paddy to be sampled was determined by considering several factors such as the main variety of paddy grown, main varieties in which spoilage problems incur and the main variety of rice used for making rice products. In order to ensure that samples are collected form all stages after harvest, samples were collected from farmers, wholesalers as well as from the market.

A total of 108 samples (9 samples each for 4 varieties of paddy, 4 varieties of rice and 4 types of rice products) were collected from within the study area as shown in Table 1. One kg of each sample was collected in order to obtain a representative sample.

2.4. Analysis of aflatoxin using ELISA

The quantification of aflatoxins in collected samples was analyzed based on a competitive enzyme linked immunoassay method using Bio-Shield B1 5 ELISA test kit (Catalog No. B5048/B5096, ProGnosis Biotech, Larissa, Greece).

2.4.1. Sample preparation

The collected samples were prepared as per the guidelines for Bio-Shield Total 5 ELISA test kit. A representative sample from the collected sample was grinded to the particle size of fine instant coffee (50% passes through a 20 mesh screen).

20 g of ground portion of the sample was taken and 100 ml of 70% methanol was added to the sample followed by mixing in a blender for 2 min.

The particulate matters were then allowed to settle followed by filtering through a Whatman No. 1 filter paper to collect about 5-10 ml filtrate.

2.4.2. Assay procedure

All the reagents were brought to room temperature before use and the washing buffer (phosphate buffered saline with 0.05% tween 20) was reconstituted to 1 liter volume with deionized water.

Appropriate number of dilution microwells (green colored) was placed in a microwell holder for the aflatoxin standards and sample to be tested. Equal number of antibody coated microtiter was placed in another microwell holder. Two hundred µl of Aflatoxin Horse Radish Peroxidase gd (HRP)- conjugate solution was dispensed into each dilution microwell followed by addition of 100 µl of each aflatoxin standard and samples to appropriate dilution microwells. These two solutions were mixed by priming pipetting for at least three times. Using new pipette tips for each, 100 µl of the contents from each dilution well was transferred to a corresponding antibody-coated microtiter well. The well was then incubated at room temperature for 15 min. The liquid from each of this antibody-coated microtiter well was aspirated into the sink and the microwell holder was tapped upside down strongly on an absorbent paper to ensure complete removal of liquid from the wells.

The wells were then washed by filling each well with diluted PBS-Tween wash buffer and the wells were again aspirated into the sink. Finally, the microwells were again tapped upside down onto an absorbent towel to remove residual water completely.

This washing process was carried out for a total of eight times. 100 µl of substrate reagent (stabilized tetramethylbenzidine) was then again added to each microwell followed by incubation for 5 min under darkness. Finally, 100 µl of stop solution was added to each microwell and the optical density of each microwell was read with a microtiter plate reader using a 450 nm filter.

2.4.3. Calculation of aflatoxin concentration

The average absorbance values for each set of duplicate standards and samples were calculated and the % binding was calculated as:

% binding = (Standard or sample absorbance/ Standard 1 absorbance) ×100 (1)

Standard curve was plotted by taking the aflatoxin concentration of standards against their respective % bindings. Finally, by using the % binding of samples, corresponding aflatoxin concentration in the samples was calculated.

2.5. Data Analysis

Each sample was analyzed in triplicates and data were tabulated for comparison and represented graphically using Microsoft Excel-2016 (16.2614.2625) Copyright Microsoft Corporation.

The values were subjected to one way Analysis of Variance (ANOVA) at 5% level of significance by using GenStat Discovery edition 12, GenStat Producer Library Release PL20.1. (Copyright 2009, VSN International Ltd). One sample t-test was performed to determine if there is any significant difference between sample values and maximum AFB1 permitted by Nepal Government, WHO and EU.

3. Results

3.1. Survey report

Through survey, it was found that the major varieties of paddy cultivated in the study area were *Ranjit* (26.61%), *Sarana* (22.22%), *Sukkha* (5.56%) and NR-2167 (13.89%) while the remaining 31.72% belonged to other varieties. When asked about problems incurred during storage of paddy, 22.22% respondents reported fungal attack, 11.11% reported change in color, 8.09% reported development of off odor while 58.58% reported other problems. Similarly, during storage of rice, 53.85% and 35.10% respondents reported the major problem to be fungal attack and appearance of lumps respectively while 11.05% of respondents reported other problems. Among the respondents, 75% reported that they use pesticides. Likewise, when asked about good agricultural practices, 76.92% respondents replied that they had no idea, 15.38% were found to have little idea whereas only 7.69% had knowledge regarding good agricultural practices.

In addition to this, just 4% of the respondents knew about aflatoxin, 9% had very little knowledge while 87% of them were unaware about aflatoxin.

3.2. Calibration curve

The kit used in this study for determining the level of AFB1 contamination in paddy was provided with aflatoxin standards with the concentration of 0.0 ppb, 0.2 ppb, 0.5 ppb, 1.0 ppb, 2.0 ppb and 4.0 ppb. On determining the optical densities for each standard by using 450 nm filter, a standard curve was obtained as shown in Fig. 1.

3.3. Aflatoxin contamination

The mean level of aflatoxin contamination in paddy samples was found to be 1.43 µg/kg and the values ranged from 0.915 µg/kg for *Sarana* paddy to 2.34 µg/kg for *Sukkha* paddy (Fig. 2(A)). Highest levels of contamination was observed in Ranjit paddy (2.02 ±0.022 µg/kg) while the least in Sarana paddy (0.923 ±0.010 µg/kg). Similarly, the AFB1 levels in different rice varieties collected is shown in Fig. 2(B). The highest levels were witnessed in *Ranjit* variety (2.02±0.022 µg/kg) while the least in parboiled rice (0.66±0.147 µg/kg), with a mean AFB1 level of 1.41 µg/kg. Likewise, the rice products collected (flaked rice and puffed rice) were found to contain a mean value of 1.64 µg AFB1 per kg sample and the values ranged from 0.79 µg to 2.35 µg per kg sample in Sarana bitten rice and *Ranjit* bitten rice respectively, which is shown in Fig 2(C). On average, highest levels were detected in *Ranjit* bitten rice (2.31±0.052 µg/kg) while the least in *Sarana* bitten rice (1.062±0.313 µg/kg).

Table 1. Collection of samples from farmers, wholesalers and market

From Farmer	From Wholesaler	From Market
Ranjit paddy	*Sarana* paddy	Parboiled rice
Ranjit rice	*Sukkha* paddy	*Sarana* puffed rice
RP paddy	*Sarana* rice	*Ranjit* beaten rice
Sarana beaten rice	*Mansoori* rice	*Mansoori* puffed rice

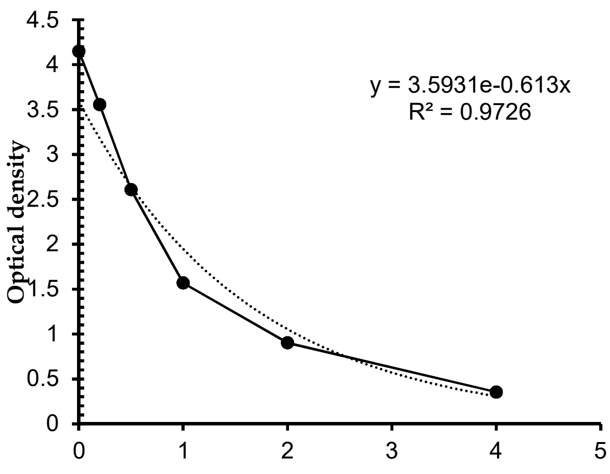

Figure 1. Calibration curve for determination of AFB1 by ELISA

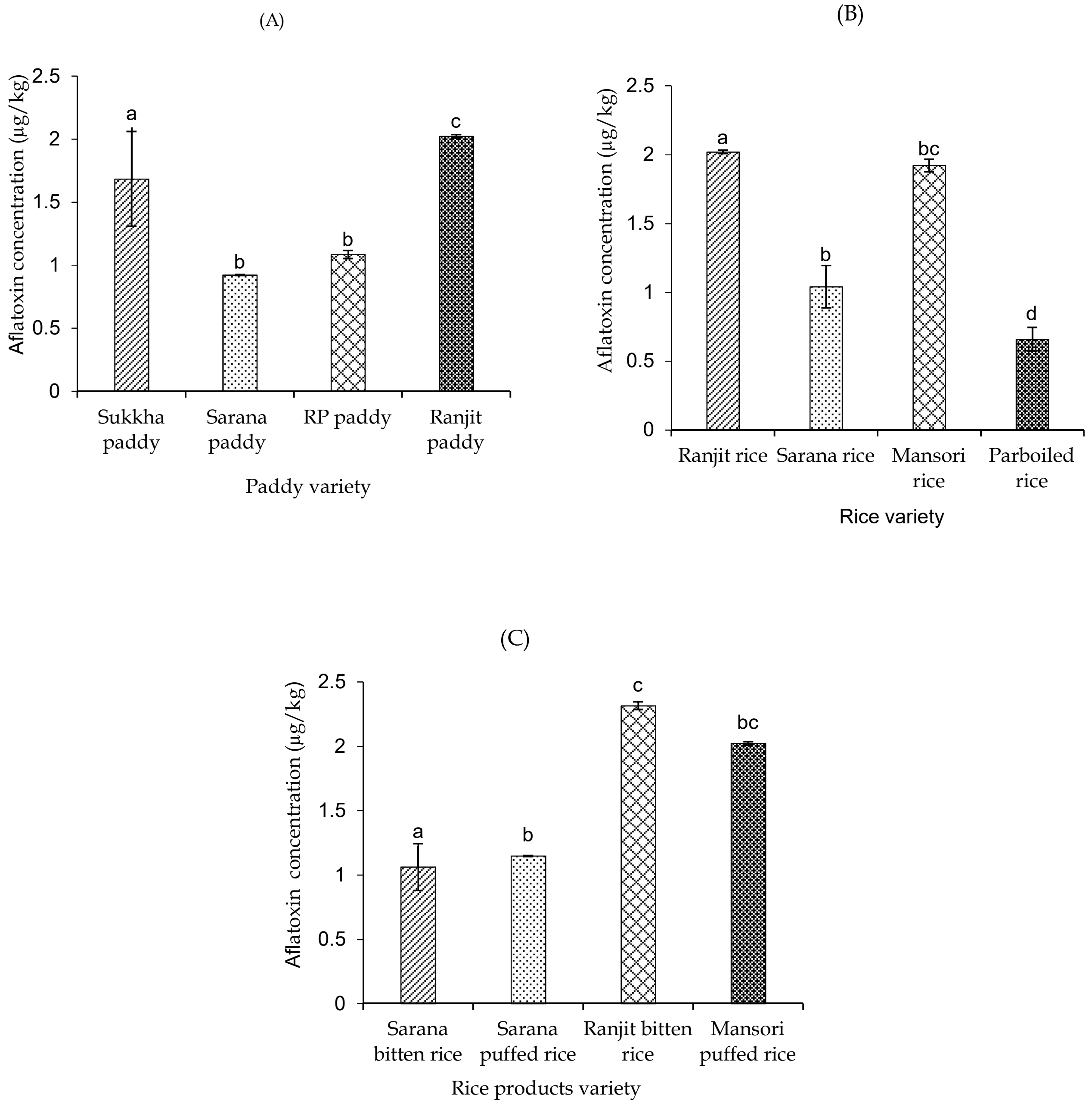

Figure 2. Level of AFB1 contamination in paddy (A), rice (B) and rice products (C) collected from Baniyani, Jhapa

*Plotted values are the means of nine samples. Vertical error bars represent ± standard deviations. Values on top of the bars bearing different superscripts are significantly different at 5% level of significance.

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4.Discussion

The survey reports indicated that fungal contamination is a major issue incurred by farmers during storage of paddy and rice. The level of awareness among farmers regarding the eradication of this problem was also inadequate. High incidence of such fungal contaminations might be a result of climatic conditions (high temperature, humidity and heavy rainfall, which are optimal for fungal growth) as well as poor storage conditions.

Among the samples collected, mean contamination levels in rice products (puffed rice and bitten rice) was relatively higher than in paddy and rice samples. Although, it is reported that mycotoxin levels can be reduced during thermal processing by utilizing temperatures above 150°C (48), the flaked rice and puffed rice samples contained higher AFB1 levels than rice and rice products, even though they are exposed to high temperatures during their manufacture. Since poor storage conditions promote aflatoxin production in rice, it may be an indication that low quality rice is used for the production of rice products.

Statistical analysis showed that there was a significant difference in the level of aflatoxin contamination among different paddy varieties collected ($P<0.05$). This indicates varying level of susceptibility of different paddy varieties towards fungal attack, although intensive research is need for further clarification. The level of aflatoxin contamination in paddy samples was found to be lower in comparison to the findings of Iqbal et al., (2012) who reported an average of 16.35 µg/kg AFB1 in paddy from Pakistan. Similarly, very high level of AFB1 contamination was reported by Reddy et al. (31) who observed the level to vary from 0.1 to 308 µg/kg in paddy samples collected from different states across India. On the other hand, the values observed in this study were higher than the findings in Brazil where contamination up to 0.551 µg/kg paddy sample was reported (45).The concentration of AFB1 in all of the paddy samples was found to be significantly lower ($p<0.05$) than the permitted limits by Nepal Government (<20 µg/kg) and by WHO (<30 µg/kg). But one of the sample of *Sukha* paddy and all of the samples of *Ranjit* paddy had AFB1 above the permitted limits set by the European Union (<2 µg/kg). Thus, despite of little knowledge about aflatoxins and good manufacturing practices, present status of AFB1 in paddy in Jhapa district was found to be safe on comparing with the standards set by Nepal Government and WHO.

Similarly, the AFB1 levels in different rice varieties was found to differ significantly ($p<0.05$). Comparable level of contamination of AFB1 was observed by Katsurayama et al. (45) where the contamination was found to be up to 2.826 µg/kg in rice samples collected from Brazil. Roy et al., (2012) reported aflatoxin contamination in rice collected from Bangladesh to be below 0.9 µg/kg (46) which is significantly lower than our findings. Similarly, the amount of AFB1 was reported to be in the range 0.1-46.2 µg/kg in rice samples collected from Swedish retail markets (47) and in the range 1.07-24.65 µg/kg in brown rice sample collected from Pakistan (28) which is much higher than our findings.

In addition to this, Toteja et al. (34) studied AFB1 contamination in parboiled rice collected from different states of India and found the levels to be as high as 361 µg/kg which is significantly higher than our findings for parboiled rice. On comparing the amount of AFB1 in collected rice samples with the regulatory standards, AFB1 contamination in all of the rice samples was found to be significantly lower ($p<0.05$) than the permitted limits by Nepal Government (<20 µg/kg) and by WHO (<30 µg/kg). But one of the samples of *Mansoori* rice and all of the samples of *Ranjit* rice had AFB1 above 2 µg/kg which is the maximum permissible level of AFB1 in the EU. Taking the standards set by Nepal Government and WHO for comparison of AFB1 all of the rice samples can be considered safe for consumption.

Also, there was a significant difference ($p<0.05$) in AFB1 concentrations among different rice product samples. Reiter et al. (49) collected 5 puffed rice samples from the market in Austria and found out that none of the samples contained aflatoxin residues. Similarly, in another study by Katsurayama et al., flaked rice samples collected from Brazil also showed no detectable amounts of aflatoxins (45). Likewise, AFB1 as high as 10.2 µg/kg was reported in sweet puffed rice balls collected from markets in Pakistan (50). The concentration of AFB1 in the entire rice product samples collected were significantly lower ($p<0.05$) than the maximum permitted levels allocated by Nepal Government (<20 µg/kg) and by WHO (<30 µg/kg). Thus, the products were found to be acceptable on comparing with Nepal standards and the standards set by WHO. But three samples of *Ranjit* beaten rice and *Mansoori* puffed rice had AFB1 above 2 µg/kg which is the maximum permitted levels permitted by the European Union. *Ranjit* beaten rice samples were found to have significantly high AFB1 levels than the EU permitted limit.

5. Conclusions

The present study was conducted to determine the status of AFB1 in rice and rice products from Jhapa district of Nepal. All of the samples collected were found to be contaminated with AFB1. The highest level of contamination was recorded in *Ranjit* variety in case of paddy, rice as well as in rice products. Although, all of the samples complied with the standards set by Nepal Government and by WHO, 1 sample of *Sukkha* paddy, 3 samples of *Ranjit* paddy, 1 sample of *Mansoori* rice, 3 samples of *Ranjit* rice, 3 samples of *Ranjit* beaten rice and 3 samples of *Mansoori* puffed rice failed to comply with the standards set by European Union. Presence of aflatoxin in day to day food commodities like rice is a major threat to consumer's health. Thus, routine monitoring of aflatoxins levels in rice needs to be performed by food manufacturers as well as regulatory bodies to ensure consumer safety.

Conflict of interest

The authors declare no conflict of interest.

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