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Occurrences of Aflatoxins in Selected Rice Production Areas in Malaysia

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ABSTRACT

Mycotoxins contamination in rice often occurs in the field prior to harvest, processing and storage. The possible coexistence of four mycotoxins in rice including aflatoxin B1, B2, G1 and G2 were investigated in the present study. The samples of rice were collected in 33 rice production areas of Padiberas Nasional Berhad (BERNAS) Rice Factories (KBB) in Malaysia. These toxins were extracted from the samples, purified and finally quantified by high performance liquid chromatography (HPLC) with fluorimetry detection. Contamination of aflatoxin G1 was found to be the most prominent, followed by B2, G2 and B1. The coexistence of aflatoxin G1 in rice was found in the highest percentage. Occurrences of aflatoxin B1, B2, G1 and G2 at 36 rice production areas showed a significant difference between east, north and central Peninsular Malaysia ranging from 0.45 to 5.98 ng/g. The mean values for aflatoxin B1, B2, G1 and G2 were 3.234, 4.697, 6.588 and 0.773 ng/g, respectively. The total aflatoxin levels for Kuching (7.61 ng/g), KBB Sungai Limau (7.33 ng/g) and KBB Paya Keladi (6.59 ng/g) were found to exceed the levels set by the European Commission (EC) as well as by the Malaysia Food Regulation 1985. Moreover, the B1 levels for Kuching (3.56 ng/g) and Sarikei (2.42 ng/g) samples also exceeded the level set for B1 by the European Commission. Nevertheless, the findings showed that the concentrations of aflatoxin in most of the rice production areas in Malaysia are still below the permissible limit under both the EC and Malaysia.

Keywords: Aflatoxin B1, aflatoxin B2, aflatoxin G1, aflatoxin G2.

INTRODUCTION

Mycotoxins produced by moulds of genera *Aspergillus, Penicillium* and *Fusarium* occur both in temperate and tropical regions of the world, depending on the species of fungi (Kumar et al., 2008; Polak-Śliwińska and Paszczyk, 2021). Nowadays, more than 400 mycotoxins are known and their number is constantly increasing, as well as the legislative provisions taken to control their presence in food and feed (Reddy et al., 2010; Pickova et al., 2021). Furthermore, significant economic losses (25%) occur in global agricultural commodities because of mycotoxin contamination (Mamo et al., 2020; Muhialdin et al., 2020).

Aflatoxins namely B1, B2, G1 and G2 are highly toxic and produced by fungi in/on foods and feeds. Aflatoxins have been associated with various diseases, such as aflatoxicosis in livestock, domestic animals and humans throughout the world (Negash, 2018; Jiang et al., 2021). Aflatoxin has received enormous attention than any other mycotoxins because of their demonstration of potent carcinogenic effect

in susceptible laboratory animals and acute toxicological effects in humans (Benkerroum, 2020; Yang et al., 2022).

Certain environmental factors influence the occurrence of aflatoxins. The severity of contamination will depend on geographical location, agricultural and agronomic practices, and the susceptibility of commodities to fungal invasion during pre-harvest, storage, and/or processing periods (Parimi et al., 2018; Amai et al., 2021). Hence, many countries have taken steps to limit the exposure of aflatoxins by setting up regulations on their limits in food and feed (Guo et al., 2021; Nada et al., 2022).

Aflatoxin is carcinogenic and has been correlated with hepatocellular carcinoma (HCC) incidence (Mortezazadeh and Gholami-Borujeni, 2022). In 2013, Mohd-Redzwan and co-researchers reported that some of the national contaminated foods had levels greater than the permissible limit of 5 ng/g adopted by the Malaysia Food Regulation 1985. An urge has been initiated to determine the occurrence of aflatoxins in the national staple food. Hence, the objective of this study was to determine the occurrence of aflatoxin B_1, B_2, G_1, and G_2 in rice production areas of Padi Beras Nasional Berhad (BERNAS) (BERNAS, 2015) within Peninsular and East of Malaysia.

MATERIALS AND METHODS

Sampling area

Rice samples were collected directly from different factories of BERNAS, Malaysia's state trading enterprise (STE) in the international rice market (BERNAS, 2015). The BERNAS Rice Factories (Kilang Beras BERNAS; KBB) involved were from 22 locations in Peninsular Malaysia, five locations in Sabah and nine locations in Sarawak. For East Malaysia, the samples were purchased from the community market in each state of major rice production areas with reference to the registered rice mills and warehouse from BERNAS. The locations of rice samples were shown in Table 1.

Chemicals and reagents

Mix standards of aflatoxins (B_1, B_2, G_1 and G_2) were purchased from Sigma-Aldrich (USA). Methanol and acetonitrile were of HPLC-grade and purchased from Sigma-Aldrich. Water was purified in a Purelab Classic ELGA system (UK). All other reagents were of analytical grade available from Merck (Germany). Stock solutions containing four aflatoxins in concentrations ranging from (0.3 to 1 µg/mL) were used to prepare series concentration by diluting solutions with methanol.

Method validation

A series of method validation tests was performed during each analysis. Stock solutions at different concentrations (0.5, 1.5, 10.0, 20.0, 50.0, 80.0 and 100.0 ng/g) were prepared for each aflatoxin. The calibration curves were developed for each series of analysis and spiked samples (1.0, 5.0 and 10.0 ng/g) were used to check the recoveries. The limit of detection (LOD) and limit of quantification (LOQ) values were obtained through the determination of the lowest concentration of the samples that can be detected by HPLC through comparison with the acceptable standard concentrations by the European Commission (European Commission, 2016).

Table 1. Selected rice production areas of BERNAS Rice Factories (KBB)

Sabah	Sarawak	Peninsular Malaysia
Keningau	Kuching	KBB Kerpan
Kota Belud	Sri Aman	KBB Kuala Perlis
Kudat (Kota Marudu)	Miri	KBB Sungai Besar
Sandakan	Sibu	KBB Paya Keladi
Tawau	Limbang	KBB Kuala Rompin
	Sarikei	KBB Changkat Lada
	Samarahan	KBB Sungai Manik
	Betong Sarawak	KBB Sungai Renggam
	Mukah	KBB Tumpat
		KBB Sungai Limau
		KBB Pering
		KBB Bukit Besar
		KBB Teluk Kechai
		KBB Kangkong
		KBB Jerlum
		KBB Tiram Jaya
		KBB Sekinchan
		KBB Tanjung Karang
		KBB Bukit Raya
		KBB Sungai Baru
		KBB Megat Dewa
		KBB Guar

Extraction and clean up

The determination of aflatoxin in rice samples was performed according to the AOAC method (McCleary et al., 2000). The contents of the plates sampled for aflatoxin analysis were milled with Warring blender (Milford, MA, USA) to obtain finely ground rice. Subsequently, a 50 g sub-sample of ground rice and 5 g NaCl were homogenised in a Warring blender (USA) with 100 mL MeOH/H_2O (80/20 v/v) for 1 min. After filtration of the mixture through a 0.45 µm Whatman membrane filter (Whatman, UK), a 15 mL aliquot of the extract was transferred into a beaker and 30 mL of deionised H_2O was added. The diluted aliquot was then filtered using a Whatman glass microfiber filter (Whatman GF/B, UK). A 15 mL aliquot of the filtrate was introduced into AflaTestimmunoaffinity columns in the capacity of 100 ng (AflaTestWB, USA) at a flow rate of 1 mL/min using adjustable air pump (USA). After washing twice with 10 mL deionised H_2O, the aflatoxins were eluted with 1.5 mL MeOH followed by 1.5 mL of deionised H_20 at a flow rate of 1 mL/min. Thereafter, the extracts were collected in glass vials and 20 µL of each extract was directly injected into HPLC system. The HPLC system (Waters 600, Milford, MA, USA) consisted of a Waters TM 600E pump, an auto-sampler (Waters 717), in-line degasser (Waters AF), and fluorescence detector (Waters 2475). The system was controlled by the Empower software (Waters).

Separation of the aflatoxins was achieved using the isocratic mode with a mobile phase consisting of water/methanol/acetonitrile (59:27:14, v/v) running through C-18 analytical column (Purospher @STAR, RP-18, 5 µm to 250 x 4 mm, LichroCart, Merck, Germany). The intensity of signals was enhanced using a photochemical reactor (Model PHRED, USA) which was placed between the LC-column and a fluorescence detector. The detector (Waters 2475) was operated at emission and excitation wavelengths of 365 nm and of 435 nm, respectively. Samples were spiked with aflatoxin (AFB_1, AFB_2, AFG_1 and AFG_2)

standard to achieve 1.0 to 10.0 ng/g. The spiked samples were then kept for 2 h at room temperature (26 - 30°C) for the removal of solvent through evaporation before extraction and HPLC analysis. Recovery analyses were carried out on each working day per batch of samples running. The recovery was calculated by comparing the concentration of the spiked samples with those of blank samples

RESULTS AND DISCUSSION

The occurrence of aflatoxins was evaluated and quantified from the samples collected in selected paddy production areas in Malaysia. Figure 1 showed representative calibration curves for the identification and quantification of individual aflatoxin compounds namely aflatoxin B1, B2, G1 and G2. The identification of the aflatoxin compounds found in a sample was based on their retention times and fluorescence detector in the HPLC system. All calibration curves were observed to show good concordance with the respective aflatoxin standards.

Table 2 showed the LOD and LOQ values of detectable samples for the detection limits of aflatoxin B_1, B_2, G_1, and G_2. The results revealed that the detection limits for aflatoxin B_1, B_2, G_1 and G_2 were 1.82, 1.52, 2.20 and 2.61 ng/g, respectively. The LOD and LOQ values for aflatoxin B1, B2, G1 and G2 were 1.819 and 3.234, 1.521 and 4.697, 2.207 and 6.588, and 2.610 and 0.773 ng/g, respectively.

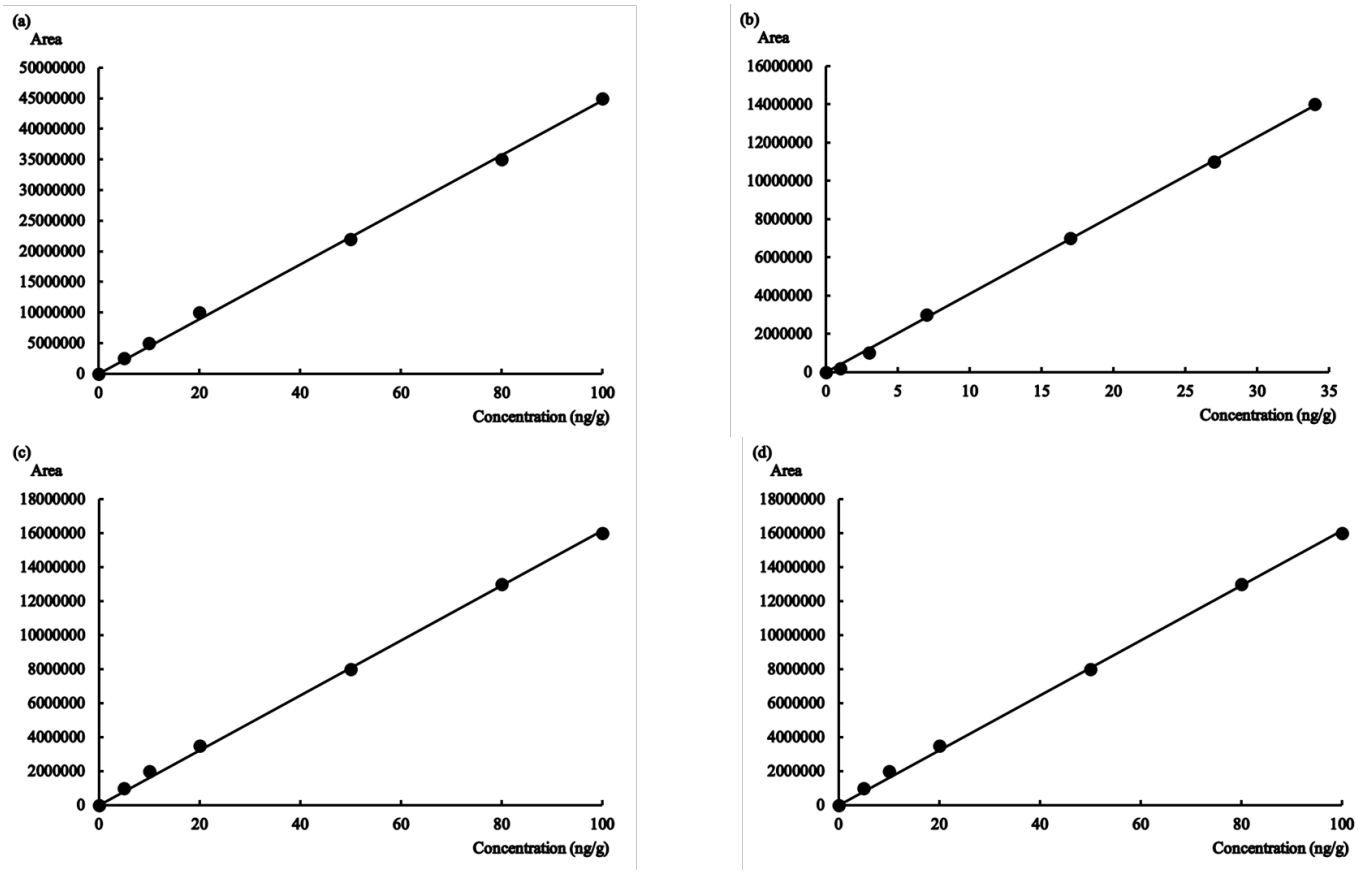

Figure 1. Calibration curves for aflatoxins B1 (a), B2 (b), G1(c) and G2 (d)

Table 2. LOD and LOQ values of detectable samples for aflatoxins

Aflatoxin	Detectable Samples	Quantifiable Samples	LOD (ng/g)	LOQ (ng/g)
B1	36	5	1.819	3.234
B2	36	5	1.521	4.697
G1	36	6	2.207	6.588
G2	36	5	2.610	0.773

Table 3 showed the average recoveries of aflatoxins and relative standard deviations (RSD). The recovery was more than 90% for most of the compounds for aflatoxin B1 (92.5%), B2 (94.6%), G1 (97.3%), and G2 (90.2%). Good recoveries of spiked samples demonstrated the accuracy of the methods used in the present study.

Table 3. Average recoveries of aflatoxins and relative standard deviations

Aflatoxin	1.00 ng/kg	5.00 ng/kg	10.00 ng/kg	RSD
B1	98.90%	93.47%	95.92%	4.29
B2	100.99%	91.55%	93.88%	3.74
G1	90.23%	95.88%	92.41%	3.62
G2	89.72%	99.04%	95.89%	5.58

The results of the aflatoxins in Table 4 showed that 13 areas from 36 selected areas (36.11%) were contaminated with aflatoxins in the range of 0.73 to 7.61 ng/g. Moreover, samples from Sarawak were contaminated in most cases of aflatoxin (B1, B_2, G_1, and G_2) incidents compared to Sabah and Peninsular Malaysia. Aflatoxins were most abundant in the Kuching area where B1, B2 and G1 were found to be 3.56, 1.27 and 3.25 ng/g, which contributed to the highest concentration of total aflatoxin of 7.61 ng/g compared to other rice production areas conducted in the present study. Sandakan and KBB Sungai Limau showed 2.30 and 7.33 ng/g of total aflatoxins occurrence representing the highest incidents in the area of Sabah and Peninsular regions, respectively.

The results were compared with the level set in the European Commission (EC) of 2 ng/g for aflatoxin B1 and 4 ng/g total aflatoxin which has been established in all cereals and all products derived from cereals, except for maize (European Commission, 2010). Three sites were found to exceed the total aflatoxin level set in the EC. Apart from Kuching (7.61 ng/g), the other two areas were KBB Sungai Limau (7.33 ng/g) and KBB Paya Keladi (6.59 ng/g). Furthermore, Kuching (7.61 ng/g), KBB Sungai Limau (7.33 ng/g) and KBB Paya Keladi (6.59 ng/g) samples also exceeded the level set by the Malaysia Food Regulation 1985 at 5 ng/g (Mohd-Redzwan et al., 2013). All sites in Sabah were well below the permissible limit of total aflatoxin.

The highest occurrence of individual aflatoxin namely G2 was 5.98 ± 1.23 (KBB Paya Keladi), followed by B2 at 4.37 ± 0.66 (KBB Sungai Limau), B1 and G1 at 3.56 ± 0.35 and 3.25 ± 0.18 ng/g (Kuching), respectively. Moreover, the highest level of B1 at Kuching (3.56 ng/g) and Sarikei (2.42 ng/g) were found to exceed the 2 ng/g for aflatoxin B1 level set by the European Commission (2010). The highest total aflatoxin and aflatoxin B1 in Kuching should be raised as an alarming situation since both values exceeded the permitted limits. Nevertheless, overall, only 8.3% (3/36) sampling sites revealed aflatoxin levels above the limit set by the European and Malaysian regulations.

In previous a study, Reiter et al. (2010) reported that 81 rice samples were purchased from different markets in Vienna and were analysed for their aflatoxin content. Different samples including basmati rice, whole grain rice, long grain rice, short grain rice as well as puffed rice were investigated. The results revealed that 24 out of 81 samples contained aflatoxins in the range of 0.45 to 9.86 ng/g for aflatoxin B1 and 1.5 ng/g for aflatoxin B2. Neither aflatoxin G1 nor G2 was detected in any sample. Three samples exceeded

the maximum level for B1concentration set by EC which were 2.16, 2.85 and 9.86 ng/g. The three organically produced rice samples only contained traces of aflatoxins.

Table 4. Occurrences of aflatoxins in selected rice production areas in Malaysia

Location	Aflatoxin (ng/g)				
	B1	B2	G1	G2	Total
Kuching	3.56 ± 0.35	1.27 ± 0.20	3.25 ± 0.18	BDL	7.61 ± 2.16
Sri Aman	BDL	0.85 ± 0.58	2.67 ± 0.06	BDL	2.88 ± 0.48
Miri	1.67 ± 0.20	BDL	BDL	BDL	1.52 ± 0.19
Sibu	1.21 ± 0.58	BDL	2.44 ± 0.28	BDL	3.50 ± 0.79
Limbang	1.21 ± 0.0.35	0.45 ± 0.16	2.58 ± 0.06	BDL	3.87 ± 1.06
Sarikei	2.42 ± 0.24	1.56 ± 0.38	BDL	BDL	3.55 ± 0.44
Tawau	BDL	BDL	BDL	BDL	BDL
Keningau	BDL	BDL	BDL	BDL	BDL
Kota Belud	BDL	BDL	BDL	BDL	BDL
Kudat (Kota Marudu)	BDL	BDL	BDL	BDL	BDL
Sandakan	BDL	BDL	2.31 ± 0.21	BDL	2.30 ± 0.45
KBB Kerpan	BDL	BDL	BDL	BDL	BDL
KBB Sungai Limau	BDL	4.37 ± 0.66	2.72 ± 0.18	BDL	7.33 ± 1.57
KBB Pering	BDL	BDL	BDL	BDL	BDL
KBB Bukit Besar	BDL	BDL	BDL	BDL	BDL
KBB Teluk Kechai	BDL	BDL	BDL	BDL	BDL
KBB Kangkong	BDL	BDL	BDL	BDL	BDL
KBB Jerlum	BDL	BDL	BDL	1.36 ± 0.14	1.39 ± 0.47
KBB Sungai Baru	BDL	BDL	BDL	BDL	BDL
KBB Megat Dewa	BDL	BDL	BDL	BDL	BDL
KBB Guar	BDL	BDL	BDL	2.64 ± 0.42	2.15 ± 0.67
KBB Kuala Perlis	BDL	BDL	BDL	BDL	BDL
KBB Sungai Besar	BDL	BDL	BDL	BDL	BDL
KBB Paya Keladi	BDL	BDL	BDL	5.98 ± 1.23	6.59 ± 1.05
Samarahan	BDL	BDL	BDL	1.15 ± 0.47	0.73 ± 0.34
KBB Kuala Rompin	BDL	BDL	BDL	BDL	BDL
KBB Changkat Lada	BDL	BDL	BDL	2.28 ± 0.25	2.16 ± 0.59
KBB Sungai Manik	BDL	BDL	BDL	BDL	BDL
Betong Sarawak	BDL	BDL	BDL	BDL	BDL
Mukah	BDL	BDL	BDL	BDL	BDL
KBB Sungai Renggam	BDL	BDL	BDL	BDL	BDL
KBB Tumpat	BDL	BDL	BDL	BDL	BDL
KBB Tiram Jaya	BDL	BDL	BDL	BDL	BDL
KBB Sekinchan	BDL	BDL	BDL	BDL	BDL
KBB Tanjung Karang	BDL	BDL	BDL	BDL	BDL
KBB Bukit Raya	BDL	BDL	BDL	BDL	BDL

BDL: Below Detection Limit

Previous literature also reported that 21.4% of paddy production areas in India are contaminated by aflatoxin which indicates some environmental conditions, geographical location, agricultural and agronomic practices as well as susceptibility of commodities to fungal invasion during pre-harvest, storage and/or processing period will affect the existing or contamination of this dangerous compound (Shephard, 2003; Ali, 2019).

CONCLUSIONS

In conclusion, this study gave the impression that most of the rice production areas in Malaysia are still below the permissible limit under the European Commission and the Malaysia Food Regulation 1985. However, the aflatoxin contaminations reported in three sites namely Kuching, KBB Sungai Limau and KBB Paya Keladi should be addressed by the appropriate authorities. Hence, as a precautionary measure, aflatoxin analyses should be proposed as a mandatory test before a production batch is distributed for sale purposes to ensure the quality and safety of the food is maintained.

AUTHORS CONTRIBUTION

KK conceived and designed the work. KK, NN, RI, RS and SC performed the analysis. KK wrote the paper, and checked and approved the submission.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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