Acta Med. Okayama, 2016
Vol. 70, No. 3, pp. 167–173

Acta Medica Okayama
http://escholarship.lib.okayama-u.ac.jp/amo/

Original Article

Aflatoxins in Rice Artificially Contaminated with Aflatoxin-producing *Aspergillus flavus* under Natural Storage in Japan

Satoshi Sugihara[a], Hiroyuki Doi[a], Masahiko Kato[b], Yoshihiro Mitoh[c], Toshihide Tsuda[d], and Satoru Ikeda[e]*

Departments of [a]Epidemiology, and [c]Oral Physiology, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, [e]Department of Medical Technology, Graduate School of Health Sciences, Okayama University, Okayama 700–8558, Japan, [b]Department of Animal Pharmaceutical Sciences, Kyushu University of Health and Welfare, Nobeoka, Miyazaki 882–8508, Japan, [d]Department of Human Ecology, Okayama University Graduate School of Environmental and Life Science, Okayama 700–8530, Japan

Aflatoxin (AFT) contamination is frequent in foods grown in tropical regions, including rice. Although AFTs are generally not found in temperate-region foods, global warming has affected typical temperate-region climates, potentially permitting the contamination of foods with AFT-producing *Aspergillus flavus* (*A. flavus*). Here we investigated the AFT production in rice during storage under natural climate conditions in Japan. We examined AFTs in brown rice and rough rice artificially contaminated with *A. flavus* for 1 year in Japan, and we subjected AFTs in white rice to the same treatment in airtight containers and examined the samples in warm and cold seasons, simulating the storage of white rice in general households. In the brown rice, AFTs increased after 2 months (March) and peaked after 9 months (October). The AFT contamination in the rough rice was minimal. After the polishing and cooking of the brown rice, AFTs were undetectable. In the white rice stored in airtight containers, AFTs increased after 1 month (August) and peaked after 2 months (September). Minimal AFTs were detected in the cold season. Thus, AFT contamination in rice may occur in temperate regions following *A. flavus* contamination. The storage of rice as rough rice could provide be useful for avoiding AFT contamination.

Key words: *Aspergillus flavus*, aflatoxin, rice, temperate region, storage

Aflatoxins (AFTs), a common mycotoxin, are secondary metabolites produced by fungi such as *Aspergillus flavus* (*A. flavus*) and *A. parasiticus*, which exist mainly in tropical and subtropical soils. AFTs have been shown to cause cytotoxicity [1], hepatocarcinoma [2], and genetic abnormalities [3] and are therefore classified as a group 1 human carcinogen [4]. There are four major subtypes of AFTs: B1, B2, G1, and G2 [5]. Among these, AFT B1 is the most toxic [6]. Because there is no effective method for the removal of AFTs, grains contaminated with AFTs and foods/feed stuffs made from materials contaminated with AFTs are usually discarded. Rice is a staple food for more than half of the global population, including the Japanese population [Food and Agriculture Organization of the United Nations, International Year of Rice 2004: Rice and Us. Available from <http://www.fao.org/rice2004/en/rice-us.htm> (Sep 3, 2015, date last accessed)]; hence, in

Received September 15, 2015; accepted December 2, 2015.
*Corresponding author. Phone:+81–86–225–6898; Fax:+81–86–235–6898
E-mail:sikeda@md.okayama-u.ac.jp (S. Ikeda)

Conflict of Interest Disclosures: No potential conflict of interest relevant to this article was reported.

addition to its negative impact on health, AFT contamination is also a major economic problem [7].

Few reports have described AFT-producing fungi and toxins in Japan. AFT-producing fungi have been detected in the soil in Japan's Nansei Islands [8, 9] and in the southern Kyusyu region of Japan [10]. In 2011, 70 μg/kg AFT B1 was found in brown rice from a rice field at the University of Miyazaki [11]. Although these regions are located in the southern region of Japan, *A. flavus* may be widely distributed throughout Japan in the future. Indeed, many studies have already shown that climate change is affecting the distribution of wild species' populations. For example, the distributions of fungi and oomycetes are expanding relatively quickly at 7 and 6 km/year, respectively [12]. Although there are no reports describing the widespread distribution of *A. flavus* in Japan, given global warming [Japan Meteorological Agency, Climate and Global Environmental Issues. Available from <http://www.jma.go.jp/jma/en/Activities/cc.html> (Sep 3, 2015, date last accessed)], it is possible that AFT production would occur in crops contaminated with AFT-producing *Aspergillus*.

In Japan, most rice is stored as brown rice in an air-conditioned environment. However, storage without air-conditioning is also common among small wholesalers or farmers and most consumers store polished white rice in airtight containers in their kitchens. The effects of these different storage conditions on AFT production in rice by contaminating *Aspergillus* are not completely understood.

In the present study, therefore, we examined AFT production in brown rice artificially contaminated during storage under natural environmental conditions in Japan. We also investigated AFT production in white rice artificially contaminated in an airtight container. Our data provide important insights into the causes of AFT contamination in rice and potential methods for reducing AFT contamination.

Materials and Methods

Rice powder contaminated with AFT-producing A. flavus. The type of rice we tested was *O. sativa* L. ssp. Japonica, purchased from a farmer in Japan's Okayama Prefecture. AFT-producing *A. flavus* (NBR 33222), which was purchased from the National Institute of Technology and Evaluation (Tokyo), produced 4 types of AFTs (B1, B2, G1, and G2). We cultured the *A. flavus* in Sabouraud bouillon medium (Sysmex, Kobe, Japan) at 25℃ for 7 days. A mixture of the culture medium (200 ml) and brown rice (1.56 kg) was incubated at 25℃ for 7 days, and the rice was then ground into a fine powder using a mill (R-2; Izumi Products, Matsumoto, Japan). The AFT concentration in the rice powder was 4.1 μg/kg.

Storage of brown rice and rough rice. The rice powder obtained as described above (25 g) was added to the sample rice (10 kg; brown rice [n = 6] or rough rice [n = 6]). The mixture was stirred well and then stored in paper bags (high-grade products accepted by the Japan Grain Inspection Association; 40 cm × 80 cm; Sanwa Co., Tokyo, Japan) under natural climate conditions in January 2013. The storage room did not have an air conditioner, and light did not enter the room. The temperature and relative humidity in the room were monitored by a hygrothermograph (TR-72i; T & D Co., Matsumoto, Japan) to evaluate the effects of climate conditions on AFT production.

AFTs in the sample rice were measured every month from January 2013 to January 2014. The initial concentration of AFTs in sample rice was set to 0.010 μg/kg, which was less than 1% of the detection limit (1.25 μg/kg) of the enzyme-linked immunosorbent assay (ELISA) kit, described below. In the control groups, AFT production was examined in brown rice (n = 1) and rough rice (n = 1) without the addition of rice powder contaminated with *A. flavus*.

Storage of white rice. In households in Japan, white rice is often stored in airtight containers for several months. For our evaluation of the AFT production under this condition, we identified the AFT levels in white rice artificially contaminated in an airtight container in warm and cold seasons. Rice powder (1.125 g) contaminated with *A. flavus* was added to white rice (450 g), and the mixture was stirred well and stored under natural climate conditions in polyethylene bags with zippers (Unipack I-4; 28 cm × 20 cm; Seisannipponsya, Tokyo, Japan) in warm (n = 6, July to October 2014) or cold (n = 6, December 2014 to March 2015) seasons. The AFT levels in the sample rice were measured every month.

Assay of AFTs. Fig. 1 is a schematic of the experiment. Briefly, in the AFT-positive cases, a portion of the sample was polished or cooked and then assayed again. In the AFT-negative cases, polishing

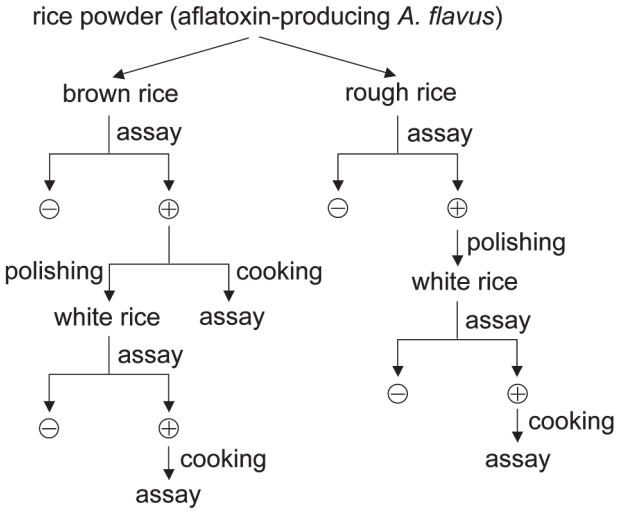

Fig. 1 Schematic overview of the study methods. Rice powder containing aflatoxin (AFT)-producing *A. flavus* was added to samples of brown rice and rough rice. The AFT production was then measured every month for up to 12 months. AFT assays in rice were performed as shown in the flowchart. −: negative AFT assay result. +: positive AFT assay result. Rough rice was processed in the same manner and was directly made into white rice without being made into brown rice. The experiments for white rice artificially contaminated with fungi in an airtight container in warm and cold seasons began from 'white rice' in this schema.

and cooking were not performed, and the assays were carried out again the next month. In addition to testing the sample rice, we examined the AFT levels in the rice bran and drainage to determine the levels of contamination in waste.

We used ELISAs to measure the AFTs, except for the AFTs in the drainage solutions after washing, which were measured by high-performance liquid chromatography (HPLC). In the test solutions of sample rice (brown rice or rough rice), randomly collected rice (150 g) was ground into a fine powder using a mill, and the powder (10 g) was mixed with 70% w/v methanol (50 ml), followed by stirring for 3 min. After 30 min, the supernatants were filtered (GF/B glass microfiber filter; Whatman, Tokyo, Japan) and used as test solutions.

ELISAs were then performed in triplicate. Standard and sample solutions (30 μl) and anti-AFT antibodies labeled with horseradish peroxidase solution (150 μl) were pre-incubated at 25℃ for 5 min, and the pre-incubation solution (100 μl) was then incubated in a 96-well flat-bottomed microtiter plate with immobilized AFT at 25℃ for 10 min. The wells were washed 3 times with washing solution, and the enzyme reaction was then performed by incubation with the substrate solution (100 μl) at 25℃ for 5 min. The reaction was terminated by the addition of stop solution (100 μl). The absorbance at 450 nm was measured with an ELISA plate reader (Model 680; Bio-Rad, Hercules, CA, USA). Polishing of the brown rice and rough rice was performed using 2 rice polishers: MR-E500 (Twinbird Co., Tsubame, Japan) and HL-40 (Agritecno Yazaki Co., Himeji, Japan), respectively. Rice cleaning was carried out using a rice washer (KOM-3; Izumi Products Co., Matsumoto, Japan). Rice was cooked in a rice cooker (NM-SR03A; KN Chiyoda Co., Tokyo, Japan).

The determination of the AFT levels by HPLC was conducted as described [Ministry of Health, Labour and Welfare, 2012. Assay of total aflatoxins. Available from <http://www.mhlw.go.jp/topics/yunyu/other/2011/dl/110816-3.pdf> (in Japanese) (Sep 3, 2015, date last accessed)]. The test solutions were prepared as follows. The water used for washing the rice was filtered (5C filter paper), and the supernatants (10 ml) were loaded on an immunoaffinity column (AflaStar R; Romer Labs, Union, MO, USA), and the column was washed with distilled water. AFTs were extracted by adding acetonitrile (3 ml) into the column, and they were derivatized with trifluoroacetate (0.1 ml) after evaporation to dryness.

The trifluoroacetate solution was then used as the test solution and was loaded onto the HPLC system equipped with a Wakosil-II 5C18 HG column (5 μm particles; 250 × 4.6 mm). The mobile phase was composed of acetonitrile: water: methanol (1 : 6 : 3, v/v/v) at the flow rate of 1 ml/min. The fluorescence intensity of the AFTs was measured with an excitation filter at 365 nm and emission filter at 450 nm. The amount of AFTs was calculated on the basis of the standard curve obtained in the AFT assays. All values are expressed as the mean ± SEM.

Results

AFTs in brown rice and rough rice in Japan. Fig. 2 shows the air temperature and relative humidity in the storage room used in this study. The range of highest and lowest air temperatures were 10.4–32.6℃ and 4.2–26.7℃, respectively, and the range of

highest and lowest relative humidities were 69–82% and 32–47%, respectively. Overall, the air temperatures and relative humidities appeared to be different from those of, for example, Ho Chi Minh City in Vietnam [World Meteorological Organization, Monthly Average Temperature and Rainfall in Ho Chi Minh City 30-year period. Available from <http://world-weather.wmo.int/en/home.html> (Sep 3, 2015, date last accessed)], which is within the tropical climate region. However, for air temperatures from July to September, the ranges of the highest and lowest values were similar to those of Ho Chi Minh City, suggesting that this climate was suitable for growth of the *A. flavus* and AFT production.

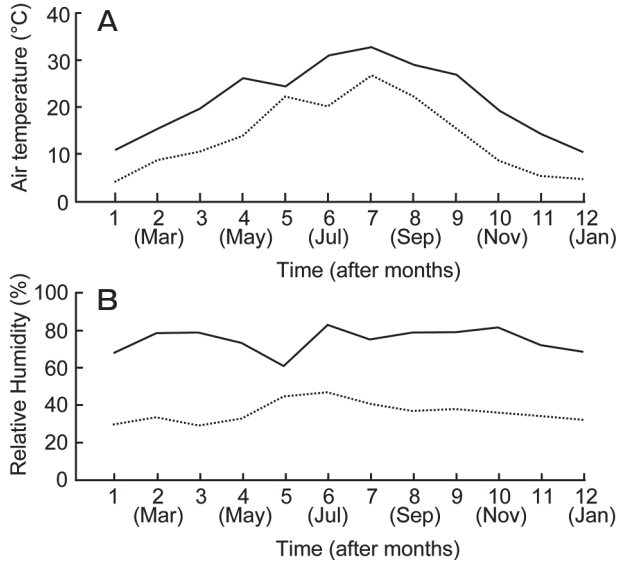

Fig. 2 Air temperatures and relative humidity in the storage room. The air temperatures (**A**) and relative humidity (**B**) in the storage room were measured from January 2013 to January 2014. The highest and lowest values for the temperature and relative humidity for each month are shown as solid and dotted lines, respectively.

Fig. 3 shows the mean concentration of AFTs in the brown rice (n = 6) and rough rice (n = 6) after artificial contamination with *A. flavus* during storage under natural climate conditions for up to 12 months before polishing (Fig. 3A) and after polishing (Fig. 3B). In the brown rice before polishing (Fig. 3A), AFTs were detected after 2 months (March: 6/6 samples) and reached a maximum level after 9 months (October: 6/6; 131.6 ± 27.2 μg/kg; range 214.2–45.3), after which the AFT levels remained constant. After cooking, the maximum concentration of the AFTs was 7.6 μg/kg in the brown rice (data not shown), which was lower than the maximum regulation value (10 μg/kg) set by the Food Sanitation Act in Japan. In the brown rice after polishing (Fig. 3B), the AFT concentrations in the majority of the samples decreased to < 10 μg/kg. Residual AFTs after polishing were completely removed by cooking in all samples (data not shown). Because AFTs are stable at high temperatures and cannot be broken down by heating during

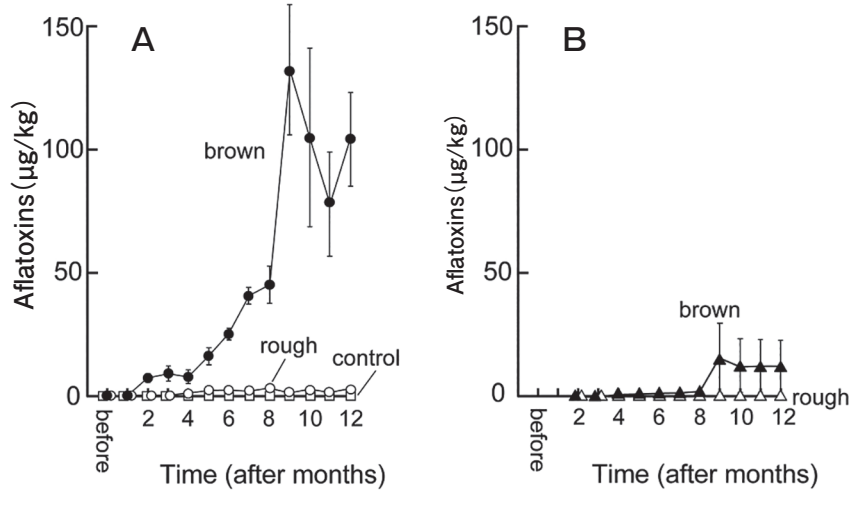

Fig. 3 AFTs in brown rice and rough rice containing AFT-producing *A. flavus*. AFTs were measured in brown rice (n = 6) and rough rice (n = 6) containing AFT-producing *A. flavus* before polishing (**A**) and after polishing (**B**). (**A**) Closed circles: brown rice. Open circles: rough rice. Control: open squares. (**B**) Closed triangles: brown rice. Open triangles: rough rice.

cooking [13, 14], we examined the levels of AFTs in the rice bran [15, 16]. The mean concentration of AFTs in the rice bran after 12 months was 465.5 ± 74.7 μg/kg (n = 6; range 270–702 μg/kg).

In the rough rice before polishing (Fig. 3A), AFTs were detected after 4 months (May: 2/6 samples), and no AFTs were detected in the control samples. After 12 months, the concentration of AFTs was 3.1 ± 0.9 μg/kg (5/6 samples), and AFTs became undetectable after polishing in all samples (Fig. 3B).

AFTs in the white rice in airtight containers. Fig. 4 shows the mean concentration of AFTs in the white rice contaminated with *A. flavus* in polyethylene bags for 3 months under natural conditions in warm (n = 6) and cold (n = 6) seasons. The climate conditions in the storage room during the experiment resembled those described in the Fig. 2 legend (data not shown). In the warm season (Fig. 4), the AFT concentrations began to increase after 1 month (August: 6/6 samples) and peaked after 2 months (September: n = 6; 231.4 ± 17.4 μg/kg; range 162.4–293.2).

After 2 months, the rice exhibited discoloration and an off-flavor; the smell was still present after cooking, and the taste of the rice was awful. After cooking, the AFT concentration exceeded the limit (n = 6; 29.1 ± 3.2 μg/kg; range 23.5–40.4). Moreover, considerable amounts of AFTs were detected in the water used for washing the rice (n = 6; 39.8 ± 3.5 μg/kg; range 31.9–55.3). In the cold season (Fig. 4, n = 6), AFTs were not detected for 3 months, but were detected at relatively low levels after 4 months (data not shown; April, n = 6; 1.2 ± 0.4 μg/kg; range ND to 2.3). At this time, the smell and taste of the cooked rice were normal.

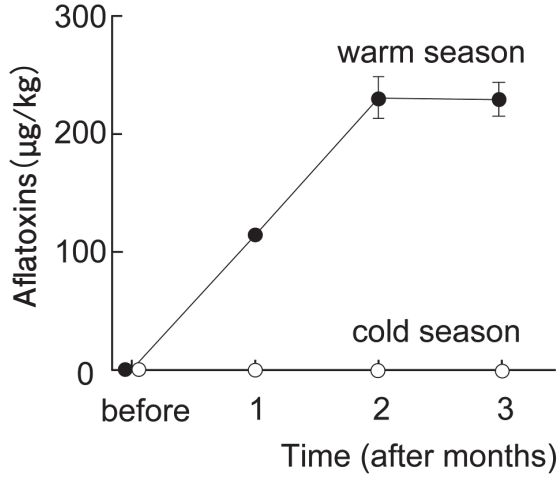

Fig. 4 AFTs in white rice artificially contaminated with AFT-producing *A. flavus* in an airtight container. Closed circles, warm season; Open circles, cold season.

Discussion

The present study is the first report to show that AFT contamination in rice occurred during storage under natural environmental conditions in a temperate region (Japan) following artificial contamination with *A. flavus* in rice. The AFT contamination in the rough rice was minimal. AFTs were also detected in the white rice contaminated with *A. flavus* in airtight containers during storage under natural climate conditions in the warm season. These data have important implications in the development of techniques to prevent AFT contamination.

In the rough rice, the AFT levels in all of the tested samples remained lower than 10 μg/kg (the maximum regulation value in Japan) throughout the experimental period. We suspect that the reason for this minimal contamination is that the rice husks of the rough rice prevented *A. flavus* from invading inside the rice [17]. Using this advantage of rough rice, farmers in the Mekong Delta of Vietnam store their rice as rough rice. In fact, we could detect no AFTs in rough rice (n = 13) from Tram Chim district, which is located at the same latitude as Ho Chi Minh City and has a similar climate, although we did not have detailed information about the storage condition or soil contamination with the toxin for that area. Thus, the storage of rice as rough rice can prevent AFT contamination without the need for specialized facilities and cooling equipment, although more space is needed because of the increased volume of rice husks. For rice that is imported, its transportation as rough rice is recommended instead of the currently used transportation as white rice.

The results of the present study demonstrated that AFT contamination in rice occurred under natural climate conditions in Japan after contamination with *A. flavus*. We observed that the AFT production increased with the increase in air temperature, suggesting that high air temperature is likely to be an important factor mediating AFT production. Moreover, a previous

study showed that the AFT production in rice is limited at temperatures below approx. 20℃ with a relative humidity of 85% [18, 19]. In our present experiment, mean air temperatures > 20℃ as calculated from the highest and lowest values shown in Fig. 2 were observed from May to October, during which the AFT production was active.

However, we observed some AFT production under cooler conditions before May, which was inconsistent with previous reports describing these limiting conditions; this suggested that factors other than air temperature, as described below, are also involved in the production of toxins. In the sample rice, rice damaged by insect pests or by the rice husker was retained. Additionally, the ventilation was not optimal because the samples were contained in paper bags. These factors may explain the observed increases in AFT production and were not considered in previous studies. In general, damaged grains may permit increased fungal invasion and further induce AFT production in various grains [20–22], including rice [15, 23, 24]. Thus, the variability in our data may be explained by the presence of damaged rice in our rice samples. Consistent with this, a study of peanut kernels showed that the limiting air temperature was lower for damaged peanut kernels than that for undamaged peanut kernels [25].

We also observed that the AFT production was increased during the warm season in the white rice contaminated with AFT-producing *A. flavus* during storage in airtight containers. The surface inside the polyethylene bags after 1 month was slightly clouded with vapor during the warm season but not during the cold season, suggesting that the relative humidity in the container was higher than the humidity in the room during the warm season, which may promote the production of AFTs. Thus, in addition to high temperature, the moisture from the sample rice may also have contributed to AFT production. Accordingly, white rice should be eaten soon after its purchase, particularly in the summer.

Taken together, our data suggest that the climate conditions, storage time, ventilation, and damage to rice may have affected the AFT production in this study. However, these factors are not specific for AFT contamination (with the exception of damaged rice), and they are also critical factors for contamination with general mold. Therefore, the prevention of AFT contamination may be realized by using methods for avoiding contamination with general mold, even in the presence of fungi. In rice in particular, it is simple to increase the number of washes to remove additional contamination, and this method could be applied in the general households. Moreover, as the current trend of using brown rice for health reasons increases [26, 27], it will be necessary to examine the effects of the storage of brown rice in greater detail.

In conclusion, AFT contamination in rice can occur under natural climate conditions in temperate regions such as Japan when AFT-producing fungi are present. The storage of rice as rough rice may be beneficial for the prevention of AFT contamination. We also have data that AFTs could accumulate in imported agricultural products such as corn and nuts under inadequate storage conditions, even when the AFT levels are lower than maximum regulation value. Increased surveillance of such agricultural products is necessary.

Acknowledgments. The authors thank Dr. N. Miyatake (Department of Hygiene, Faculty of Medicine, Kagawa University, Japan) for helpful comments on the manuscripts and encouragement. This work was conducted with the support of a Grant-in Aid for Scientific Research (no. 26670261) from the Ministry of Education, Culture, Science, Sports and Technology of Japan.

References

1. Al-Hammadi S, Marzouqi F, Al-Mansouri A, Shahin A, Al-Shamsi M, Mensah-Brown E and Souid AK: The cytotoxicity of aflatoxin B1 in human lymphocytes. Sultan Qaboos Univ Med J (2014) 14: e65–e71.
2. Massey TE, Stewart RK, Daniels JM and Liu L: Biochemical and molecular aspects of mammalian susceptibility to aflatoxin B1 carcinogenicity. Proc Soc Exp Biol Med (1995) 208: 213–227.
3. Gross-Steinmeyer K and Eaton DL: Dietary modulation of the biotransformation and genotoxicity of aflatoxin B1. Toxicology (2012) 299: 69–79.
4. International Agency for Research on Cancer: Some naturally occurring substances: food items and constituents, heterocyclic aromatic amines and mycotoxins; In IARC Monographs on the Evaluation of Carcinogenic Risks to Human, IARC Press, Lyon (1993) pp 245–251.
5. Pitt JI: Toxigenic fungi and mycotoxins. Br Med Bull (2000) 56: 184–192.
6. Yunus AW, Razzazi-Fazeli E and Bohm J: Aflatoxin B(1) in affecting broiler's performance, immunity, and gastrointestinal tract: a review of history and contemporary issues. Toxins (Basel) (2011) 3: 566–590.
7. Wu F, Bhatnagar D, Bui-Klimke T, Carbone I, Hellmich R, Munkvold G, Paul P, Payne G and Takle E: Climate change impacts on mycotoxin risks in US maize. World Mycotoxin J (2011) 4: 79–93.

8. Manabe M, Tsuruta O, Tanaka K and Matsuura S: Distribution of aflatoxin-producing fungi in soil in Japan. Trans. Mycol. Soc. Japan (1976) 17: 436–444 (in Japanese).
9. Takahashi H, Kamimura H and Ichinoe M: Distribution of aflatoxin-producing Aspergillus flavus and Aspergillus parasiticus in sugarcane fields in the southernmost islands of Japan. J Food Prot (2004) 67: 90–95.
10. Okazaki H, Saito M and Tsuruta O: Population levels of Aspergillus flavus and A. parasiticus in field soils in two areas of Kyushu district. Ann Phytopath Soc Japan (1992) 58: 208–213.
11. Asahi Shimbun morning edition, February 17, 2011, Mold toxin from the rice (in Japanese).
12. Bebber DP, Ramotowski MAT and Gurr SJ: Crop pests and pathogens move polewards in a warming world. Nature Clim Change (2013) 3: 985–988.
13. Milani, J and Maleki G: Effects of processing on mycotoxin stability in cereals. J Sci Food Agric (2014) 94: 2372–2375.
14. Scott PM: Effects of food processing on mycotoxins. J Food Protect (1984) 47: 489–499.
15. Castells M, Ramos A, SanchisV and Marín S: Distribution of total aflatoxins in milled fractions of hulled rice. J Agric Food Chem (2007) 55: 2760–2764.
16. Trucksess MW, Abbas HK, Weaver CM and Shier WT: Distribution of aflatoxins in shelling and milling fractions of naturally contaminated rice. Food Addit Contam Part A (2011) 28: 1076–1082.
17. Ma JF and Yamaji N: A cooperative system of silicon transport in plants. Trends Plant Sci (2015) 20: 435–442.
18. Choi S, Jun H, Bang J, Chung SH, Kim Y, Kim BS, Kim H, Beuchat LR and Ryu JH: Behaviour of Aspergillus flavus and Fusarium graminearum on rice as affected by degree of milling, temperature, and relative humidity during storage. Food Microbiol (2015) 46: 307–313.
19. Mousa W, Ghazali FM, Jinap S, Ghazali HM and Radu S: Modeling growth rate and assessing aflatoxins production by Aspergillus flavus as a function of water activity and temperature on polished and brown rice. J Food Sci (2013) 78: M56–M63.
20. Raghavender CR, Reddy BN and Shobharani G: Aflatoxin contamination of pearl millet during field and storage conditions with reference to stage of grain maturation and insect damage. Mycotoxin Res (2007) 23: 199–209.
21. Reddy BN, Brijitha N and Raghavender CR: Aflatoxin contamination in insect damaged seeds of horsegram under storage. Mycotoxin Res (2005) 21: 187–191.
22. Wood GE: Mycotoxins in foods and feeds in the United States. J Anim Sci (1992) 70: 3941–3949.
23. Osman N, Abdelgadir A, Moss M and Bener A: Aflatoxin contamination of rice in the United Arab Emirates. Mycotoxin Res (1999) 15: 39–44.
24. Siruguri V, Kumar PU, Raghu P, Rao MVV, Sesikeran B, Toteja GS, Gupta P, Rao S, Satyanarayana K, Katoch VM, Bharaj TS, Mangat GS, Sharma N, Sandhu JS, Bhargav VK and Rani S: Aflatoxin contamination in stored rice variety PAU 201 collected from Punjab, India Indian J Med Res (2012) 136: 89–97.
25. Diener UL and Davis ND: Limiting temperature and relative humidity for growth and production of aflatoxin and free fatty acids by Aspergillus flavus in sterile peanuts. J Am Oil Chem Soc (1967) 44: 259–263.
26. Kozuka C, Sunagawa S, Ueda R, Higa M, Tanaka H, C. Shimizu-Okabe, Ishiuchi S Takayama C, Matsushita M, Tsutsui M, Miyazaki J, Oyadomari S, Shimabukuro M and Masuzaki H: γ-Oryzanol protects pancreatic β-cells against endoplasmic reticulum stress in male mice. Endocrinology (2015) 156: 1242–1250.
27. Kozuka C, Yabiku K, Sunagawa S, Ueda R, Taira S, Ohshiro H, Ikema T, Yamakawa K, Higa M, Tanaka H, Takayama C, Matsushita M, Oyadomari S, Shimabukuro M and Masuzaki H: Brown rice and its component, γ-oryzanol, attenuate the preference for high-fat diet by decreasing hypothalamic endoplasmic reticulum stress in mice. Diabetes (2012) 61: 3084–3093.