Aloha International Journal of Health Advancement (AIJHA)
ISSN 2621-8224
Volume 2 Number 12, December 2019
http://journal.aloha.academy/index.php/aijha
RESEARCH
DOI: http://dx.doi.org/10.33846/aijha21203

The Aflatoxin Contamination in Spices Sold on the Jember Market

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Submitted: November 1, 2019 -Revised: December 11, 2019 -Accepted: December 21, 2019 -Published: December 31, 2019

ABSTRACT

Aflatoxin is a secondary metabolite of the fungus *Aspergillus flavus and Aspergillus parasiticus*, the toxin is able to cause health problems in humans and animals that suffer sympthom of aflatoxin called mycotoxicosis. Both types of fungus can survive at optimally temperature of 36-38^{o}C and moisture above 85%. *Aspergillus* species may contaminated some foods such as wheat, rice, corn, beans, chilies, and spices. Aflatoxin have six types, namely Aflatoxin B1, B2, G1, G2, M1 and M2. AFB1 is the most toxic among the six types of aflatoxin, it's induce cancer by affecting Deoxyribonucleic Acid (DNA) genetic code. The aim of this research was to analyze the types and levels of aflatoxin especially spices that provided in the traditional market and supermarkets in Jember area. This research applied with experimental analytic. The population was spices that belong in all of traditional markets and supermarkets in Jember area. The sample was certain spices (onion, turmeric, pepper) in the 3 traditional markets and 3 supermarkets selected by purposive sampling techniques. This research was done at Central Laboratory Agro Industry Bogor on July to September 2019. The variable of this research was aflatoxin contamination inside of spices. Aflatoxin analytic performed by High Performance Liquid Chromatography (HPLC) instruments. The results of this research showed that found some of samples that has been analyzed, the samples of pepper (C4) was taken in Supermarket 'X' has the highest contaminated aflatoxin B1 was 45,35 ppb and aflatoxin G1 was 50,74 ppb. Therefore, its recommended for Supermarket is to increased monitoring of temperature and humidity, especially at the storage of spices.
Keyword: aflatoxin; fungus; traditional market; spices; supermarket

INTRODUCTION

Natural foods are basically safe to consume. However, inappropriate food processing often produces harmful products for health. The rise of such food safety issues can occur because of climate changes[(1).] The uncertain climate changes can support the growth of microbes polluting food products. Moreover, improper storage and drying processes can cause *Aspergillus* fungus to grow on condiments and spices[(2).] Spices are mostly produced in high humidity countries, which are the most conducive environment for fungus to grow[(3).]

Since Indonesia is located in the equator, which has a tropical climate, mycotoxin can contaminate food commodities easily[(4).] Mycotoxin seriously affects human health, both acutely and chronically[(5).] There are two kinds of mycotoxin, which become a crucial concern – aflatoxin and ochratoxin. Aflatoxin is the most dangerous mycotoxin for health. The aflatoxicosis fatal outbreak indicates it as the cause of the improper post-harvest foodstuff treatments that tropical countries have reported[(6).]

The consumption of high aflatoxin-contaminated foods can cause acute aflatoxicosis and hepatotoxicity manifestation. In more chronical issues, death cases can occur due to fulminant liver failure[(7).] Aflatoxin B1 is suspected to cause cancer by inducting *Deoxyribonucleic Acid* (DNA) which leads to the target cell changes impairing the DNA threads; therefore, cancer may occur. Human cancer is caused by the mutated tumor antigen p53 (protein 53) – the guardian genome. This tumor antigen plays a crucial role in preventing cells from genetic mutations due to DNA impairments, such as preventing transversion mutation from codon 249 guanine (G) into thymine (T) which causes Hepatocellular Carcinoma (HCC) by 50%[(8,9).]

In Kenya, there was an aflatoxicosis case causing 317 people poisoned and 125 death casualties because of the high consumption of aflatoxin-tainted corns in 2014. Moreover, in 2013, Romania, Serbia, and Croatia reported that there were milk products contaminated with aflatoxin across the countries[(10).] The previous research findings indicate that, from 350 corn samples available on markets, 192 samples with aflatoxin level >20 ppb, 121 samples with aflatoxin level > 100 ppb, and 24 samples with aflatoxin level >1000 ppb[(11)].

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Another previous research findings show that there were aflatoxin-contaminated peanuts sold on traditional markets and sold by merchants with aflatoxin level more than 10 ppb, even some were more than 3000 ppb[12].

Some countries regulate the maximum amount of aflatoxin content. European countries set a maximum tolerance of the total aflatoxin content in spices by 10 mg/kg and aflatoxin B1 by 5 mg/kg. In Indonesia, the National Agency of Food and Drug Control (NADFC) directs the maximum amount of the total aflatoxin by 20 ppb and aflatoxin B1 by 15 ppb in spices with its regulation Number 8 of 2018. Based on the research background above, the researcher wanted to conduct a study related to the aflatoxin contamination in spices available on traditional markets and supermarkets in the Jember Regency.

METHODS

This study applied with experimental analytic. The population was spices that belong in all of traditional markets and supermarkets in Jember area. The sample was certained of spices (onion, turmeric, pepper) in the 3 traditional markets and 3 supermarkets selected by purposive sampling techniques. Samples (100 g/sample) were collected sterilized vacumm bags and stored at 18^{o}C until used. This research was done at Central Laboratory Agro Industry Bogor on July to September 2019. Aflatoxin analytic performed by High Performance Liquid Chromatography (HPLC) instruments.

The material and tools were used in this research is spices, metanol, asetonitrile, aquadest, standart solution of aflatoxin, petri dishes, aluminum foil, plastic syringes, analytical scales, weighing bottles, ovens, desilator, filter paper, immunoafinity column, vial amber, vortex, milipore 45µm, ultrasonic, funnel, microfiber filters, watch glass, 10 mL and 25 mL serological pipettes, measuring cups, erlenmeyer 250 mL, centrifuge tubes50 mL, eppendorf pipettes 100 µL. Lichosper C-18 column (250 mm x 4.0 mm) with a particle size of 5 µm, HPLC device, UV-Vis detector.

The steps of research were:

1) The sample weighed 100 gram/sample and then extracted used extraction solvent.
2) Standard Solution Preparation
   The sample be weighed 5 gram pepper, 25 gram onion, 25 gram turmeric sample and ± 5 gram Natrium clorida powder were added to the blender, and added 125% methanol 70 ml. Then blend at high speed for 2 minutes. The solution was filtered using filter paper; the filtrate was piped as much as 15 mL and diluted with aquabidest as much as 30 mL shaken until homogeneous. A total of 15 mL of filtrate was put into the immunoaffinity column at of 1 drop per second, and 10 mL of aquabidest was put into the immunoaffinity column at a speed of 2 drops per second. After all the liquid has been pushed down using a syringe until the air was released and the liquid that is collected is discarded. Then put 1 mL of methanol into the immunoaffinity column and the droplets were collected in an amber vial.
3) Standardization of Samples
   A standard and sample of 1 mL in an amber vial was evaporated with nitrogen to dry. Then after being dried 100 ml of Trifluoroacetic Acid (TFA) was added and vortexed for 30 seconds, then incubated for 15 minutes at room temperature, then a mixture of acetonitrile-aquabidest was added as much as 900 ml (1: 9) and vortexed returned for 30 seconds. The mobile phase consisting of water, acetonitrile, and methanol is injected into column C-18 with a flow rate of 1 mL per minute and the volume of the sample injected as much as 100 µL, for further analysis used HPLC device.
4) Data Analysis
   Identification of secondary metabolite produced by fungus *Aspergillus* analyzed used HPLC. Separation of analytes was carried out using XRODSII (150 mmx 2 mm x 2.2 µm). The parameters for the analysis were determined used positive ion mode (ESI +), with the spectrum obtained in the mass range m/ z 70 – 1300 Da. The parameter used was the capillary voltage (4.5 kV), gas drying temperature (190^{o}C), drying gas flow (10 L/ minutes) and nebuized gas pressure (4 x 105 Pa). Analysis used HPLC with the electrospray operated in positive ionization mode (ESI +) used water symmetry column C18 (50 x 4.6 mm x 3.0 µm). The parameters were capillary voltage (3.0 kV), source temperature (150 ^{o}C), desolvation temperature (450 ^{o}C), cone gas flow (25 L/hour), and length of excitation and emision waves of 362 and 455 nm respectively. A mobile phase consisting of water, acetonitrile and methanol was injected to the HPLC column with a flow rate of 1 mL/ minute and volumen injected as much as 100 µL. The aflatoxin levels in sample were calculated and determined using calibration curve with a straight line (y = bx). Data obtained from the results of analysis will be presented in the frequency distributions tables and narratives.

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RESULTS

The table 1 presents the measurement of temperature and humidity levels in the traditional markets and supermarkets in the Jember Regency.

Table 1. The measurement of temperature and humidity levels in the traditional markets and supermarkets in the Jember Regency data

Place	Temperature (^{o}C)	Humidity (%)
Traditional market X	23	70
Traditional market Y	27	74
Traditional market Z	26	84
Supermarket X	24	88
Supermarket Y	23	73
Supermarket Z	21	75

Table 1 showed that the temperature measurement by using a thermo-hygrometer indicated that the temperature of the traditional market was in the range of 23-27^{o}C with a 70-84% humidity level. Meanwhile, the temperature of the supermarkets was in the range of 21-24^{o}C with a 70-88% humidity level.

Below is the table presenting the identification results of the types of fungi found on spices growing on the PDA media by observing the morphology and characteristics of fungi and applying microscopic observation.

Table 2. The identification of the types of fungi growing on spices on the PDA media

Code of sample	Fungus species				
	Aspergillus flavus	*Aspergillus fumigatus*	*Aspergillus niger*	*Aspergillus terreus*	*Penicillium spp.*
A1	-	+	-	-	-
A2	-	-	+	-	-
A3	+	-	+	-	-
A4	-	-	+	-	-
A5	-	+	-	-	-
A6	-	+	-	-	-
B1	-	-	+	-	-
B2	+	-	+	-	-
B3	-	-	-	+	-
B4	-	-	+	+	-
B5	-	-	+	-	-
B6	-	+	-	+	-
C1	-	-	-	-	+
C2	+	+	-	+	-
C3	+	-	-	-	-
C4	-	-	-	+	-
C5	+	-	-	+	-
C6	-	-	-	+	-

A (onion); B (turmeric); and C (pepper)

From the research results on Table 2, there were several identified types of fungi growing on spices on the PDA media including: *Aspetgillus flavus, Aspergillus fumigatus, Aspergillus niger, Aspergillus Terreus,* and *Penicillium spp.*

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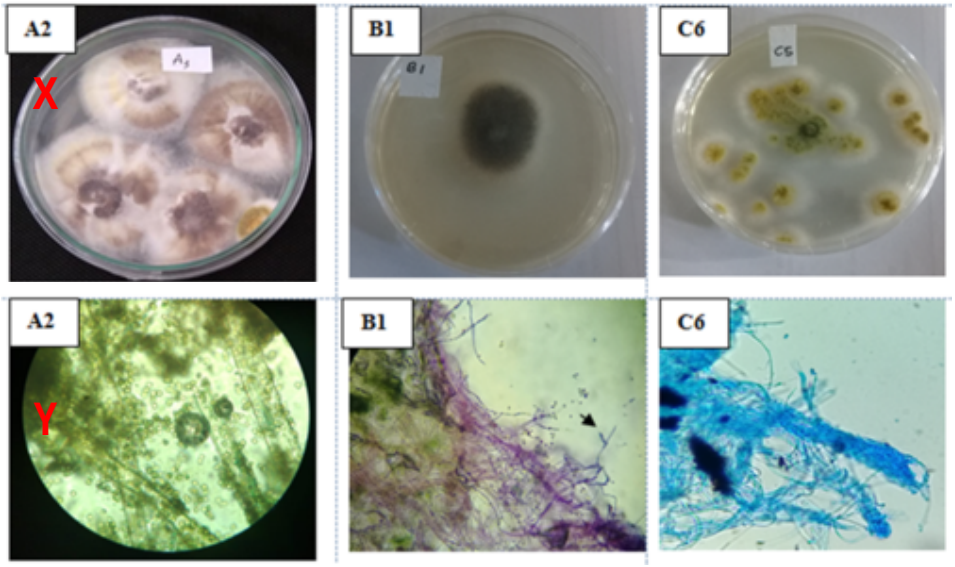

Figure 1. The code of X = the isolate observation result of the fungal colony isolates based on the morphology and characteristics of fungi; Y = the microscopic observation results of fungal isolates by 100 magnification

Table 3. The identification of the types of fungi growing on spices on the blood agar media

Code of sample	Fungus species
	Fusarium oxysprorum
A1	+
A2	+
A3	+
A4	+
A5	+
A6	+
B1	+
B2	+
B3	+
B4	+
B5	+
B6	+
C1	+
C2	+
C3	+
C4	+
C5	+
C6	+

A (onion); B (turmeric); and C (pepper)

From the research results on Table 3, the identified fungus growing on spices on the Blood Agar media was *Fusarium oxysporum*.

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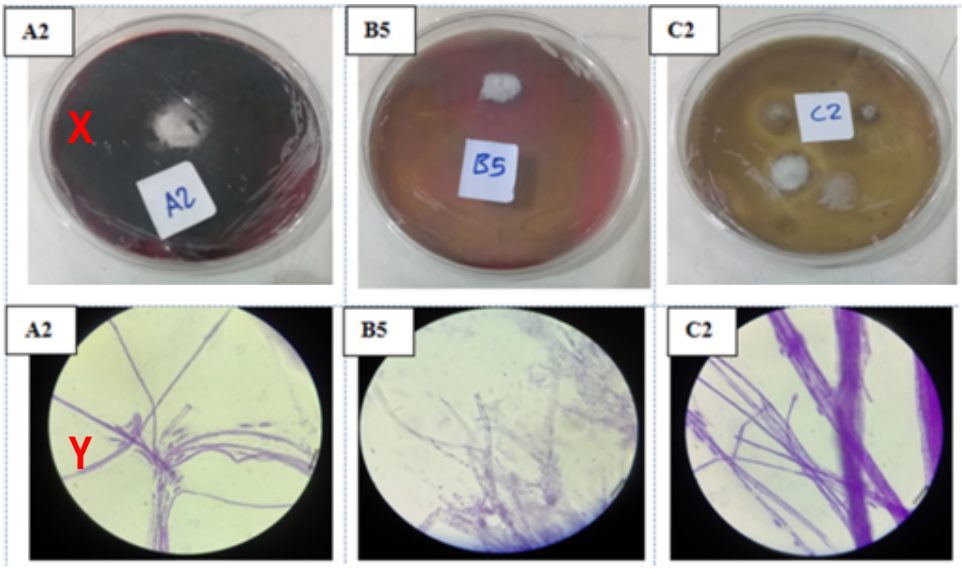

Figure 2. The code of X = the isolate observation result of the fungal colony isolates based on the morphology and characteristics of fungi; Y = the microscopic observation results of fungal isolates by 100 magnification

Below is the table of the analysis results of aflatoxin in spice samples by using the HPLC instrument under the following conditions: injection volume by 100 µL; 30 minutes retention time; and using an excitation wavelength by 362 nm and 455 nm emission.

Table 4. The Analysis Results of Aflatoxin B1, B2, G1, G2

Code of sample	Aflatoxin B1 (ppb)	Aflatoxin B2 (ppb)	Aflatoxin G1 (ppb)	Aflatoxin G2 (ppb)	Total Aflatoxin (ppb)
A1	ND	ND	0.13	ND	0.13
A2	0.14	ND	0.13	ND	0.13
A3	1.55	0.38	1.55	0.35	3.83
A4	ND	ND	ND	ND	ND
A5	ND	ND	ND	ND	ND
A6	ND	ND	ND	ND	ND
B1	ND	ND	ND	ND	ND
B2	ND	ND	ND	ND	ND
B3	ND	ND	ND	ND	ND
B4	ND	ND	ND	ND	ND
B5	1.41	0.37	1.47	0.32	3.57
B6	ND	ND	ND	ND	ND
C1	0.64	0.24	0.57	0.15	1.60
C2	0.66	0.19	0.69	ND	1.54
C3	0.67	ND	ND	ND	0.67
C4	45.35	2.08	50.74	1.18	99.35
C5	0.79	ND	ND	ND	0.79
C6	ND	ND	ND	ND	ND

ND : not detected

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DISCUSSION

Based on the analysis of aflatoxin B1, B2, G1, G2 and the total aflatoxin by applying LOQ (limit of quantification) on the 18 samples: 0,5 µg/kg, the obtained results from the samples, code A2 contains aflatoxinB1 by 0,14 ppb, code A3 by 1,55 ppb, code B5 by 1,41 ppb, code C1 by 0,64 ppb, code C2 by 0,66 ppb, code C3 by 0,67 ppb, code C4 by 45,35 ppb, and code C5 by 0,79 ppb. From all samples applied with the analysis of the aflatoxin B1, B2, G1, G2, and the totalaflatoxin, the results expose that the sample C4 contains the highest aflatoxin contamination level, with the total aflatoxin is 99.35 ppb.

The pepper sample with code C4 is the most contaminated spice samples gathered from Supermarket X in the Jember Regency. That pepper product was processed and packaged by PT.X on October 1, 2019, and will be expired on April 1, 2020, which already follows the regulation of HACCP and GMP about food packaging, are the most aflatoxin-tainted product. It may happen because of the temperature and humidity of the storage support the Aspergillus to grow. Hence, the fungus can produce aflatoxin in a massive amount in the logarithmic phase of the fungus, where fungus can adapt to the conducive environment, which fastens the growth of the Aspergillus on a proper substrate. Besides, the sample pepper C4 is wraped with a non-vacuum plastic; consequently, the temperature and humidity in such a packaging affects the growth of the Aspergillus.

The fungus-contaminated pepper is not only found in Indonesia but also in most of the countries producing pepper because most of them still implement traditional processing in unhygienic environments [(13)]. Tosun and Recep (2013) found that black pepper sample they investigated contained aflatoxin B1 by 27.6 µg/kg [(14)]. Meanwhile, Jacxsens et al. (2016) revealed that their research samples – chilies and pepper – indicated the growth of Aspergillus flavus, A. Parasiticus, A. Niger, and Penicillium spp, in which those fungi can produce aflatoxin [(15)]. Additionally, the analysis results of 120 white pepper and black pepper samples in Malaysia, both in the form of granules or powder obtained from supermarkets (in the form of packaging) and markets (in the form retails), exposed that 47.5% were contaminated with ochratoxin A from 0.15 to 13.58 µg/g, where. Among them, 33.3% contained ochratoxin A more than the maximum limitation (5µg/g) directed by Malaysia [(16)]. In 2017, Widowati et al. discovered that all identified pepper powder samples contained Xerophilic fungi, which are Aspergillus candida, A. ochraceus, A. fumigatus, Eurotium herbariorum, A. tamarii, E. Chevalieri, A. penicilodes, A. niger, and A. oryzae [(17)].

The turmeric sample with the code B5 contains aflatoxin by 1, 41 ppb. The sample B5 is the turmeric samples available on the supermarket Y of in the Jember Regency. The turmeric sample B5 is contaminated with aflatoxin because the sample has been packaged since June 18, 2019, in styrofoam and wrapped with plastic where the temperature inside the packaging and the storage, and also the unhygienic post-harvest treatment done by farmers can affect the growth of Aspergillus on the turmeric. However, the turmeric sample obtained from the traditional markets does not contain any aflatoxin because the turmeric is stored in an open container. Briefly, the air cycle and the buying cycle in the traditional markets happen quicker than in supermarkets; the turmeric is not stored for a long time.

The other factor which might affect the presence of it's the anti-fungi substance in turmeric itself, which causes the Aspergillus, as the aflatoxin producer, cannot grow. Turmeric contains Curcuminoid with Curcumin Difureloylmethane as the main bioactive component, which plays as anti-fungi, anti-inflammation, antioxidant, anti-cancer, anti-protozoa, and anti-virus [(18)]. According to Ferreira, et al. (2013), inhibiting the growth of Aspergillus flavus using curcumin essential oil from turmeric (Curcuma longa L.), by giving curcuminoid from turmeric extract with the 2000 ppm concentrate can slow the growth of Aspetgillus flavus and Fusarium oxysporum[(19)]. Furthermore, Dias, et al. (2013) contend that giving essential oil from turmeric extract with the concentration of 0,5% can slow the production of aflatoxin by Aspergillus flavus by 99,99%[(20)].

From the six samples of onions available on the traditional markets and supermarkets, two samples of onions contain aflatoxin B1. Sample A2 contains aflatoxin B1 by 0.14 ppb while sample A3 contains aflatoxin B1 by 1, 55 ppb, both samples were taken from the traditional market Y and traditional market Z. The samples are contaminated with aflatoxin because of the markets' environment. Based on the temperature measurement, the market Y reach 27^{o}C and market Z 26^{o}C where the Aspergillus can produce maximum toxin in such temperature ranges. Moreover, the humidity of market Y is 74%, while market Z is 84%, which causes the Aspergillus grows and develops secondary metabolite, which is aflatoxin. The onions on the markets are stored in wooden boxes with no holes, and the volume of the onion is too massive; consequently, the air circulation cannot run well. Additionally, Aspergillum and Fusarium are pathogenic on onion, which can lead to the decay [(21)]. Chatri (2016), explains that humidity can affect the first phase of spore and pathogen growth, especially fungi such as Phytophora palmivora, P. Infestans, Fusarium oxysporum, Altenaria solani, and Oidium caricae which can proliferate in high humidity [(22)].

The research results on the onion and turmeric samples contaminated with aflatoxin B1 does not pass the maximum marker of aflatoxin contamination in spices in Indonesia issued by the Indonesian NADFC (National

Agency of Drug and Food Control). Therefore, it should be maintained in their cultivation processes form the cultivation, maintenance, harvest, and post-harvest. Furthermore, the distribution and storage processing should be controlled as well so that the quality of spices are maintained, because onions and turmeric are spices which are mainly consumed in the form of medications or food seasonings, and they are very vulnerable from decaying if the storage process is improper. In order to prevent the risks of mycotoxin contamination in spices, Nguegwouo et al. (2018) remarks that color and smell changes, which are considered as the sign of fungi growing, must be taken into account. Moreover, the duration of they are in storage must be taken into consideration because storage in room temperature in tropical regions is very suitable for fungi to grow [(23)].

The harvesting process generally conducted by farmer is by drying harvests under direct sun light without using any layer. Based on the Indonesian Ministry of Agriculture regulation number 73/Permentan/OT.140/7/2013, the drying process of spices should use layers to minimize the microbe contamination from the ground, and the absorption of water content by the spices, which cause decaying and might grow fungi. After that, the spices will be weighed and sold to middlemen to sell. The rest of the harvests which are not sold should be stored as seeds for the next cultivation. The post-harvest activity is a stage of cultivating spices fitted with the harvesting age, including harvesting, transporting, sorting, drying, storing, processing, and marketing.

The storage processes carried out by the farmers in the post-harvest activity is by storing harvests in a warehouse Generally, the warehouse should be roof closed, floor-based, and has enough air vent for the air changing. The onions are stored by tying them up and hanging them, and turmerics are put straight away on a warehouse floor. Meanwhile peppers are wrapped in plastic bags and put in a warehouse. According to Safitri and Sri (2019), storing harvests in a warehouse is the crucial phase of the post-harvest activity because harvests can be vulnerably decayed by fungi [(24)]. In addition, Kader and Hussein (2009) state that besides soring harvests in a warehouse, fungi contamination in spices can occur in the cultivation process, harvesting process, and post-harvest transporting [(25)]. Farmers still conventionally use plastic webs and sacks in the packaging process, and post-harvest transporting done by open-trucks to sell and distribute harvests to seller in the markets and merchants without considering cleanliness. Moreover, the temperature changes of the environments can affect the regulator gen and production of aflatoxin from Aspergillus flavus and Aspergillus parasiticus [(26)]. The temperature and humidity which keep increasing can increase the growth of fungi and support pathogenic bacteria to grow well [(27)].

CONCLUSION

The temperature and humidity play a crucial role in promoting Aspergillus to produce aflatoxin in foodstuffs, particularly in spices. Based on the test of the 18 samples, peppers with the code C4 contains the highest aflatoxin B1 by 45, 35 ppb and the total aflatoxin by 99, 3 ppb. It is beyond the limit stipulated by the Indonesian NADFC, which are 15 ppba aflatoxin B1 and 20 ppb total aflatoxin in spices both in the solid form or powder.

The suggestion for supermarkets is to improve the temperature and humidity monitoring in spices storage area. For further researchers, a research on identifying another mycotoxin contamination like ochratoxin, where this mycotoxin is also the main concern in the agriculture and health, can be conducted. Furthermore, for the sampling technique, identifying the aflatoxin contamination in spices taken directly from harvests, markets and supermarkets, and household can be applied.

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