Screening of Toxigenic *Aspergillus flavus* Strains and Aflatoxin Content from Agricultural Commodities in Indonesia

Anidah[1,2], Winiati P. Rahayu[1,3] and Siti Nurjanah[1,3]
[1]*Department of Food Science and Technology, IPB University, Darmaga Campus Bogor 16680, Indonesia*
[2]*SEAMEO BIOTROP, Jl Raya Tajur Km. 6 Bogor 16134, Indonesia*
[3]*SEAFAST Center, IPB University, Darmaga Campus, Bogor 16680, Indonesia*

Keywords: *Aspergillus flavus*, Aflatoxin, Toxigenic *A. flavus*, HPLC.

Abstract: Infection of toxigenic *A. flavus* in agricultural commodities may result in production of aflatoxin, a mycotoxin which is genotoxic carcinogenic for humans and animals. The aims of this study were to screen toxigenic *A. flavus* strains and to determine aflatoxin content of six agricultural commodities in Indonesia. A total of 50 *A. flavus* strains were obtained from Phytopathology Laboratory, SEAMEO BIOTROP. The strains were isolated from nutmeg, corn, cacao, white pepper, coffee bean, ground peanut and peanut-cropped soil. The toxigenicity of *A. flavus* were determined bfy growth simulation on aflatoxin-inducing medium (10% coconut agar medium) followed by observation of their fluorescence using 365 nm UV light. AFB and AFG toxin produced were quantified using HPLC. The results showed that 18% (9 strains) *A. flavus* were toxigenic, which derived from nutmeg (5 strains), ground peanut (2 strains), cacao (1 strain), and peanut-cropped soil (1 strain). Six toxigenic strains produced AFB1 exceeding the Indonesian-regulatory maximum level (15 ug/kg). *A. flavus* from peanut-cropped soil (BIO 3352) produced the highest AFB1 content (90.94 ug/kg), while the other from nutmeg (BIO 3345 and BIO 33212), ground peanut (BIO 3313 and BIO 3338), and cacao (BIO 33404) had AFB1 content of 89.53, 84.24, 70.26, 40.27, and 69.06 ug/kg respectively. The producing aflatoxin capability of these strains can be potentially hazard if contaminated in agricultural commodities.

1 INTRODUCTION

Aflatoxins are secondary metabolites that produced by *Aspergillus* section *Flavi*, particulary *A. flavus* and *A. paraciticus* (Ellis *et al*, 1991). Natural occurrence of aflatoxin in agricultural product lead to severe health problems for human and livestock. Aflatoxins confirmed as a Group-1 agent which is carcinogenic to humans (IARC, 2012). Exposure to higher levels of aflatoxin increases cancer incidence, including risk of hepato-cellular carcinoma and neural tube defect (Sun *et al*, 2011 and Woo *et al,* 2011). The Food and Agricultural Organization (FAO) has been estimated that approximately 25% of crops worldwide get contaminated by mycotoxin producing fungi including *A. flavus*, that contributing to global losses of 1000 million metric tons foodstuffs each year (Bhat *et al*, 2010). The contamination by mycotoxigenic fungi can occur during harvest, postharvest, storage and transportation and causes significant economic losses yearly (Hedayati *et al*, 2007 and Nurtjahja *et al*, 2017).

Aflatoxins have a high occurrence in tropical and subtropical regions due to optimal humidity and temperature conditions for toxin production (Bhat *et al*, 2010). Contamination of aflatoxin in agricultural commodities was reported in many countries. Mandel (2005) had reported that *A. flavus* was the dominant fungi in contaminated nutmeg imported from India, Sri Lanka, Indonesia and Brazil. Aflatoxins and fumonisins were reportedly widespread in major dietary and export targeted crops such as maize and peanuts in Southern Africa (Hove *et al*, 2016; Mwalwayo *et al*, 2016). According to Davari *et al*., (2015), out of 28 strains of *A. flavus* and *A.paraciticus* isolated from 110 feed samples in northeastern Iran, 10 strains were toxigenic.

Indonesia's agricultural commodities including maize, peanut, pepper, nutmeg, and cacao have been reported contaminated by aflatoxin (Nurtjahja *et al*, 2017; Dharmaputra, 2002; Dharmaputra *et al*, 2013). About 54% of isolated fungi from stored nutmeg in North Sulawesi, was identified as *A. flavus* with highest

Anidah, ., Rahayu, W. and Nurjanah, S.
Screening of Toxigenic Aspergillus flavus Strains and Aflatoxin Content from Agricultural Commodities in Indonesia.
DOI: 10.5220/0009981200002964
In *Proceedings of the 16th ASEAN Food Conference (16th AFC 2019) - Outlook and Opportunities of Food Technology and Culinary for Tourism Industry*, pages 221-226
ISBN: 978-989-758-467-1

level of AFB1 and total aflatoxin content of 1.63 ppb and 1.83 ppb respectively (Dharmaputra *et al*, 2015).

According to Indonesian Food Security Agency (Badan Ketahanan Pangan/BKP) from unpublish data noted there was still rejection for Indonesia's nutmeg commodities until 2019 due to aflatoxin contamination (Figure 1).

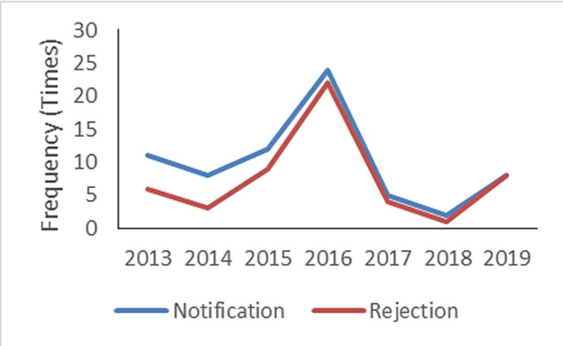

Figure 1: Notification and rejection of Indonesia's nutmeg export commodity (BKP, unpublish data).

The data from annual reports of Rapid Alert System for Food and Feed (RASFF) in the last decade showed mycotoxin alert notification in food which aflatoxin were predominantly notified each year (Table 1). Nuts and nut products were addressed as notified contaminated food product every year.

Table 1: RSAFF notification on mycotoxin and aflatoxin in food from 2010 – 2017.

Year	Notification	
	Mycotoxin	Aflatoxin
2010	688	649 (94.3%)
2011	635	585 (92.1%)
2012	528	484 (91.7%)
2013	410	341 (83.2%)
2014	359	314 (87.5%)
2015	475	421 (88.6%)
2016	489	360 (73.6%)
2017	529	416 (78.6%)

There were many researches have been conducted biological control strategy recently involving toxigenic and atoxigenic *A. flavus* to reduce aflatoxin contamination (Doner *et al*, 2003; Yin *et al*, 2009). The information about characterization toxigenic as well as atoxigenic were required as prelude for choosing suitable strains for biological control. There is minimum information about diversity of *A. flavus* isolated from agricultural product in Indonesia. The aims of this study were to screen toxigenic *A. flavus* strains and to determine aflatoxin content from six agricultural commodities in Indonesia. The information about molecular characterization can be used as further information for controlling aflatoxin contamination using screened *A. flavus*.

2 MATERIAL AND METHODS

2.1 *Aspergillus flavus* Strain

The total of 50 strains of *A. flavus* were selected randomly and were kindly provided by Fitophatology Laboratory of SEAMEO BIOTROP, Indonesia (Table 2). The strains were isolated from nutmeg, corn, cacao, white pepper, coffee bean, ground peanut and peanut-cropped soil from various regions in Indonesia. BIO 747 strain was used as positive control that can produced both AFB and AFG from previous study (Nagur *et al*, 2014). Fungal cultures were routinely subcultured on potato dextrose agar (PDA: 39 g l-1, Difco Laboratories, Sparks, USA) every two years.

2.2 Screening of Toxigenic *A. flavus* Strains

For screening toxigenicity of *A. flavus*, all strains were cultured on aflatoxin-inducing medium, 10% (v/v) coconut agar medium (CAM, 100 mL fresh shredded coconut endosperm, 900 mL distilled water, 15 g bacto agar, pH 7.0). A small amount of *A. flavus* mycelium transferred into the centre of CAM and incubated at 27^{0}C for 5 days in the dark condition. Observation of presence or absence of blue fluorescence in the agar surrounding the *A. flavus* colonies was determinated by exposing the petri dish to long-wave (365 nm) UV light and expressed as positive or negative toxigenicity. An uninoculated plate was used as reference (Nurtjahja *et al*, 2017; Davis *et al*, 1987). All the positive toxigenic strain was further confirmed for aflatoxin quantification by HPLC, along with positive control (BIO 747) and one atoxigenic strain from screening as reference.

2.3 Aflatoxin Extraction and Quantification by HPLC

Aflatoxin production simulated on 10% (v/v) coconut broth (CB, 100 mL fresh shredded coconut endosperm, 900 mL distilled water, pH 7.0) medium. As much as 2 inoculum (ϕ 5mm) of each strains were inoculated on 50 mL 10% (v/v) CB medium with continuous shaking at 100 rpm (27^{0}C, 10 days) in the dark condition.

Table 2: Expression of fluorescence and contents of aflatoxin from 50 *A. flavus* strains.

Commodities (origin)	*A. flavus* *Strains*	Fluorescence in CAM	Aflatoxin (ug/kg)				
			AFB1	AFB2	AFG1	AFG2	AF-total
Nutmeg (Manado – North Sulawesi)	BIO 3316	-	na	na	na	na	na
	BIO 3345	+	89.53	<	<	<	89.53
	BIO 33184	-	na	na	na	na	na
	BIO 33212	+	84.24	<	<	<	84.24
	BIO 33402	-	na	na	na	na	na
	BIO 33403	+	4.48	3.02	2.82	0.82	11.14
	BIO 33211	+	5.03	<	<	<	5.03
	BIO 3376	+	6.47	<	26.71	<	33.18
	BIO 33185	-	na	na	na	na	na
	BIO 35102	-	na	na	na	na	na
Coffee bean (Jember – East Java)	BIO 3314	-	na	na	na	na	na
Coffee bean (Toraja – South Sulawesi)	BIO 3384	-	na	na	na	na	na
	BIO 3393	-	na	na	na	na	na
	BIO 3394	-	na	na	na	na	na
	BIO 3396	-	na	na	na	na	na
Corn (Bogor – West Java)	BIO 3382	-	na	na	na	na	na
	BIO 3311	-	na	na	na	na	na
	BIO 35111	-	na	na	na	na	na
Cacao (Makasar – South Sulawesi)	BIO 3312	-	na	na	na	na	na
	BIO 33404	+	69.06	<	<	<	69.06
	BIO 33405	-	na	na	na	na	na
White pepper (Bogor, West Java)	BIO 3383	-	na	na	na	na	na
	BIO 3316	-	na	na	na	na	na
	BIO 25119	-	na	na	na	na	na
Ground peanut (Bogor – West Java)	BIO 3313	+	70.26	6.59	<	1.01	77.86
	BIO 3381	-	<	<	<	<	<
	BIO 3346	-	na	na	na	na	na
	BIO 3348	-	na	na	na	na	na
Ground peanut (Wonogiri – Central Java)	BIO 3342	-	na	na	na	na	na
	BIO 3324	-	na	na	na	na	na
	BIO 3334	-	na	na	na	na	na
	BIO 3338	+	40.27	<	97.28	<	137.55
	BIO 3340	-	na	na	na	na	na
	BIO 3341	-	na	na	na	na	na
	BIO 3322	-	na	na	na	na	na
	BIO 3325	-	na	na	na	na	na
	BIO 3324	-	na	na	na	na	na
Peanut-cropped soil (Wonogiri – Central Java)	BIO 3352	+	90.94	<	<	<	90.94
	BIO 3362	-	na	na	na	na	na
	BIO 3364	-	na	na	na	na	na
	BIO 3367	-	na	na	na	na	na
	BIO 3374	-	na	na	na	na	na
	BIO 3378	-	na	na	na	na	na
	BIO 3386	-	na	na	na	na	na
	BIO 3387	-	na	na	na	na	na
	BIO 3390	-	na	na	na	na	na
	BIO 3357	-	na	na	na	na	na
	BIO 3391	-	na	na	na	na	na
	BIO 3392	-	na	na	na	na	na
	BIO 3357	-	na	na	na	na	na
Toxigenic strain	BIO 747	+	74.01	<	54.05	<	128.06

(na) not applicable; (+) fluorescence observed; (-) no fluorescence observed

(<) below the LoQ, for AFB1 = 1.42 ug/kg, AFB2 = 6.72 ug/kg, AFG1 = 5.09 ug/kg, and AFG2 = 0.66 ug/kg.

Total aflatoxin was extracted from ten-days-old 10% (v/v) CB medium cultures of toxigenic strains, using AOAC method 991.3125,26. A 25 ml of filtered extract was pipetted and extracted with 5 g NaCl and 125 ml of methanol:water (70:30) ratio into blender jar, and blended for 2 minutes at maximum speed. The filtered extract (15 ml) was diluted with 30 ml of purified water into a clean vessel. The diluted extract was filtered through glass microfiber filter. A 15 ml filtered diluted extract passed completely through AflaTest affinity column (VICAM, USA) at a rate of about 1-2 drops/second and washed with 2 x 10 ml of purified water at a rate of 2 drops/second. Total aflatoxin was eluted from column with addition of 1 ml HPLC grade methanol (Merck, Germany) at rate of 1 drop/second. Eluted sample was collected in a glass cuvette and added with 1 ml deionized water. Afterward, 20 ul of eluate were injected onto HPLC.

Chromatographic analyses were performed with an Agilent 1260 Infinity Isocratic LC (Agilent Technologies, USA), equipped with Photochemical Reactor Derivatization (AURA Industries). Excitation and emission wavelengths were 365 and 465 nm respectively. A Bonclone 10u C18 Column (Phenomex, 3.9 x 150 mm) was used. The mobile phase was methanol: water (60:40) and the flow rate was 1.3 ml/min. Injection volume was 20 ul. Quantification of aflatoxin was perfomed by comparing the peak areas with the calibration curves of each aflatoxin.

3 RESULT AND DISCUSSION

The screening on aflatoxin-induced medium (CAM) was initially used to identify the aflatoxin production from 50 *A. flavus* strains by fluorescence observation, revealed that nine strains (18%) were toxigenic (Table 2).

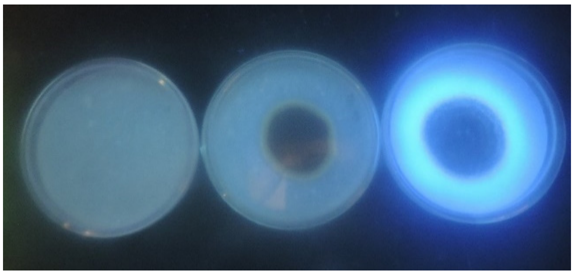

Figure 2: Fluorescence of *A. flavus* strains on 10% CAM. (Left – right); uninoculated CAM, atoxigenic strain (BIO 3381), toxigenic strain (BIO 3313).

Toxigenic strains were originated from nutmeg (56%), cacao (22%), ground peanut (11%), and peanut-cropped soil (11%). Atoxigenic strains was obtained from coffee bean, corn, and pepper. Blue fluorescence was observed from outside of the colony in CAM from eight strains, meanwhile fluorescence was not seen either from uninoculated media or atoxigenic strain (Figure 2). The naturally presence of toxigenic and atoxigenic *A. flavus* have been reported in many studies. Wei *et al*, (2014) found by UPLC detection, 76% of the 323 *A. flavus* strains isolated from peanut field in four provinces in China, were aflatoxins producer with limit of detection method was 1 µg/kg.

All the toxigenic strains, also positive and negative control strains were further confirmed by measuring AFB dan AFG content by HPLC from growth simulation in CB medium. BIO 3381 was chosen as negative control as no fluorescent observed in CAM. During the incubation process, mycelia grew on the surface of the media, while the toxin produced was dissolved in the media. The aflatoxins content determined as AFB1, AFB2, AFG1, AFG2 and total aflatoxin. The result showed all toxigenic strains produced AFB1 (Table 3). Six toxigenic strains produced AFB1 exceeding the Indonesian-regulatory maximum level (15 ug/kg). *A. flavus* from peanut-cropped soil (BIO 3352) produced the highest AFB1 content (90.94 ug/kg), while the other strains from nutmeg (BIO 3345 and BIO 33212), ground peanut (BIO 3313 and BIO 3338), and cacao (BIO 33404) had AFB1 content of 89.53, 84.24, 70.26, 40.27, and 69.06 ug/kg respectively.

Table 3: Summary of aflatoxin content of 9 toxigenic *A. flavus* strains.

Commodities	*A. flavus* Strains	Aflatoxin (ug/kg)			
		AFB1	AFB2	AFG1	AFG2
Nutmeg	BIO33212	84.24	<	<	<
	BIO33403	4.48	3.02	2.82	0.82
	BIO33211	5.03	<	<	<
	BIO3376	6.47	<	26.71	<
	BIO3345	89.53	<	<	<
Cacao	BIO33404	69.06	<	<	<
Ground peanut	BIO3313	70.26	6.59	<	1.01
	BIO3338	40.27	<	97.28	<
Peanut-cropped soil	BIO3352	90.94	<	<	<

(<) below the LoQ, for AFB1: 1.42 ug/kg, AFB2: 6.72 ug/kg, AFG1: 5.09 ug/kg, and AFG2: 0.66 ug/kg.

There was only one strain (BIO 33403) that produced all aflatoxins types. Meanwhile one strains (BIO 3313) produce all aflatoxins types except AFG1, and two strains (BIO 3338 and BIO 3376) produce AFB1 and AFG1. Five strains observed which only produced AFB1 were BIO 3345, BIO 3352, BIO 33211, BIO 33212, and BIO 33404. *A. flavus* had known as AFB producer and *A. paraciticus* as AFG producer which were determined by the color of fluorescence of the colony on 10% CAM (Nurtjahja *et al*, 2017). This study found that 44.4% strains of toxigenic *A. flavus* can produce either AFB or AFG.

In this study, strain isolated from peanut-cropped soil was the higher production of AFB1. According to Pitt (1989) in Dharmaputra *et al*., (2001), *A. flavus* and *A. paraciticus* are present in high numbers in cultivated soils. They are able to grow as commensals in developing peanut plants, and start to invade developing peanuts (Pitt *et al*., 1991). The study of soil isolates and the correlation with toxigenicity potential was reported by Dharmaputra *et al*., (2002). She reported that 44% of toxigenic *A. flavus* were identified from 48 soil sample during wet season, and 51% during dry season, in Pati regency (Central Java). Most of the toxigenic *A. flavus* produced AFB1 and AFB2 and some of them produced AFB1, AFB2, AFG1, and AFG2. Toxigenic *A. flavus* also found as much as 27.5% from 66 strains isolated from corn field soil in Iran, and only produce AFB1 or AFB1 and AFB2 (Razzaghi-Abyaneh *et al*., 2006).

4 CONCLUSIONS

Nine strains of toxigenic *A. flavus* were obtain from screening of 50 strains from 6 agriculture commodities and 1 peanut-cropped soil in Indonesia which can produced aflatoxin. It was assumed that soil from plantation could be a media for *A. flavus* infection to the plant. The result of this study gave information that toxigenic *A. flavus* strains have ability to produce aflatoxin and could be used as positive control in biological control. Further studies are needed to characterize the diversity in DNA level among the toxigenic strains. The information about molecular characterization could help to develop more effective biological control strategy.

ACKNOWLEDGEMENTS

The authors would like to acknowledge SEAMEO BIOTROP for providing financial support through Daftar Isian Pelaksanaan Anggaran (DIPA) 2019. Thanks, are also to Prof. Dr Okky Setyawati Dharmaputra and Ms. Ina Retnowati, S. Si for their *A. flavus* collection isolates.

REFERENCES

AOAC (Association of Official Analytical Chemists)., 2000. Section 49.2.18 (AOAC Method 991.31) for corn, raw peanut, and peanut butter. In Official Method of Analysis, Seventeenth Edition. Gaithersburg, MD, USA, AOAC International.

Bhat, R., Rai, R. V., & Karim, A. A., 2010. Mycotoxins in Food and Feed: Present Status and Future Concerns. Comprehensive Reviews in *Food Science and Food Safety*. (9): 57-81.

Davari E., Mohsenzadeh M., Mohammadi Gh., Rezaeian-Doloei R., 2014. Characterization of aflatoxigenic *Aspergillus flavus* and *A. paraciticus* strain isolates from animal feedstuffs in northeastern Iran. *IJVR*. 16 (2): 150-155.

Davis N.D., Iyer S.K., Diener U.L., 1987. Improved method of screening for aflatoxin with a coconut agar medium. *Appl. Environ. Microbiol*. 1987; 53(7):1593-1595.

Dharmaputra O.S., 2002. Review on aflatoxin in Indonesian food and feedstuffs and their products. *Biotropia*. 19:26 – 46.

Dharmaputra O.S., Ambarwati S., Retnowati I., Nurfadila N., 2015. Fungal infection and aflatoxin contamination in stored nutmeg (*Myristica fragnans*) kernels at various stages of delivery chain in north Sulawesi province. *Biotropia*. 22(2): 129-139.

Dharmaputra O.S., Ambarwati S., Retnowati I., Windyarani A., 2013. *Aspergillus flavus* population and aflatoxin B1 content in processed peanut product in municipality of Bogor, West Java, Indonesia. Biotropia. 20(2): 81-88.

Dharmaputra O.S., Putri A.S.R., Retnowati I., Ambarwati S., 2001. Soil mycobiota of peanut fields in Wonogiri regency, Central Java: Their effect on the growth and aflatoxin production of *Aspergillus flavus* in vitro. *Biotropia*. (17): 30-59.

Dharmaputra O.S., Retnowati I., Ambarwati S. Toxigenic *Aspergillus flavus* isolates in the soil of peanut farms in Pati regency, Central Java. 2002b. Bogor: SEAMEO BIOTROP.

Doner J.W., Cole R.J., Cinnick W.J., Daiglle D.J., McGuire M.R., Shashad B.S., 2003. Evaluation of biological control formulation to reduce aflatoxin contamination in peanuts. *Biol Control*. (38):329-339.

Ellis W.O., Smith J.P., Simpson B.K., 1991. Aflatoxin in food: occurrence, biosynthesis, effect on organism, detection and method of control. Critical Review in *Food Science and Nutrition*. (30): 403-439.

Hedayati M.T., Pascualotto A.C., Warn P.A., Bowyer P., Denning D.W., 2007. *Aspergillus flavus*: human pathogen, allergen and mycotoxin producer. Review. *Microbiologi*. 153: 1677 – 1692.

Hove M.C., Van Poucke E., Njumbe-Edjage L.K., Nyanga, S. De Saeger., 2016. Review on the natural co-occurance of AFB1 and FB1 in maize and the combined toxicity of AFB1 and FB1. *Food Control*. (59): 675-82.

IARC (International Agency for Research on Cancer), 2012. Monograph on the evaluation of carcinogenic risk to human. Chemical agent and related occupations. IARC Press. Lyon, France, Volume 100 F.

Mandel Q.A., 2005. Fungal contamination of some imported spices. *Mycopathologia*. 59: 291-8.

Mwalwayo D. S., and B. Thole., 2016. Prevalence of aflatoxin and fumonisins (B1 & B2) in maize consumed in rural Malawi. 2016. *Toxicology Reports*. (3):173–9.

Nagur K. S., Sukarno N., Listriyowati S. 2014. Identification of *Aspergillus flavus* and detection of its aflatoxin genes isolated from peanut. Biotropia. 21(1): 64-75.

Nurtjahja K., Dharmaputra O.S., Rahayu W.P., 2017. Gamma irradiation of *Aspergillus flavus* strains associated with Indonesian nutmeg (*Myristica fragrans*). *Food Sci Biotechnol*. 26(6):1755-1761.

Pitt J.I., Dyer S.K. and McCammon S., 1991. Systemic invasion of developing peanut plant by *Aspergillus flavus*. Letters in *Applied Microbiology*. (13):16-20.

Pitt, J.I., 1989. Field studies on *Aspergillus* and aflatoxins in Autralian groundnuts. In McDonald D. and Mehan V.K. Aflatoxin contamination of groundnut. Proceeding of International Workshop, 6-9 October 1989, ICRISAT Center, India. p. 223-235.

RSAFF., 2011. The Rapid Alert System for Food and Feed 2010 Annual report. European Comission.

RASFF., 2012. The Rapid Alert System for Food and Feed 2011 Annual report. European Commission.

RASFF., 2013. The Rapid Alert System for Food and Feed 2012 Annual report. European Commission.

RASFF., 2014. The Rapid Alert System for Food and Feed 2013 Annual report. European Commission.

RASFF., 2015. The Rapid Alert System for Food and Feed 2014 Annual report. European Commission.

RASFF., 2016. The Rapid Alert System for Food and Feed 2015 Annual report. European Commission.

RASFF., 2017. The Rapid Alert System for Food and Feed 2016 Annual report. European Commission.

RASFF., 2018. The Rapid Alert System for Food and Feed 2017 Annual report. European Commission.

Razzaghi-Abyaneh M., Shams-Ghahfarokhi M., Allameh A., Kazeroon-Shiri A., Ranjbar-Bahadori S., Mirzahoseini H., Rezaee M., 2006. A survey on distribution of *Aspergillus* section flavi in corn field soils in Iran: population patterns based on aflatoxins, cyclopiazonic acid and sclerotia production. *Mycopathologia*. (161): 183-192.

Sun, G., Wang, S., Hu, X., Su, J., Zhang, Y., Xie, Y., Zhang, H., Tang, L., & Wang, J. S., 2011. Co-contamination of aflatoxin B1 and fumonisin B1 in food and human dietary exposure in three areas of China. *Food Addit Contam*. Part A Chem Anal Control Expo Risk Assess. (28): 461-470.

Wei D., Zhou L., Selvaraj J.N., Zhang C., Xing F., Zhao Y., Wang Y., Liu Y., 2014. Molecular Characterization of Atoxigenic *Aspergillus flavus* Isolates Collected in China. *J. Microbiology*. 52:559-565.

Woo, L. L., Egner, P. A., Belanger, C. R., Wattanawaraporn, R., Trudel, L. J., Croy, R. G., Groopman, J. D., Essigmann, J. M., & Wogan, G. N., 2011. Aflatoxin B1-DNA adduct formation and mutagenicity in livers of neonatal male and female B6C3F1 mice. *Toxicological Sciences*. 122(1): 38 – 44.

Yin Y., Lou T., Yan L., Michailides T.J., M a Z., 2009. Molecular characterization of toxigenic and atoxigenic *Aspergillus flavus* isolates, collected from peanut fields in China. *Journal of Applied Microbiology*. (107): 1857-1865.