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Co-Occurrence of Moulds and Mycotoxins in Corn Grains Used for Animal Feeds in Malaysia

K.R.N. Reddy and B. Salleh
School of Biological Sciences, Universiti Sains Malaysia,
11800 USM, Penang, Malaysia

Abstract: About 80 corn grain samples used for animal feeds were collected from 10 states in Malaysia in order to determine the mycobiota using agar plate assay and mycotoxins (Aflatoxin B_1 and fumonisins) by ELISA. *Aspergillus flavus* (87%), *A. niger* (83%), *F. verticillioides* (47%), *F. graminearum* (43%), *F. proliferatum* (42%), *F. equisieti* (30%) and *Penicillium* sp. (5%) were the prevalent fungi in all corn samples. Aflatoxin B_1 (AFB1) could be detected in 65 (81.2%) corn samples ranging from 1.0-135 μg kg^{-1}. The 18 (22.5%) samples, out of 80 had exceeded AFB1 above the international regulatory limits of animal feeds (>20 μg kg^{-1}) ranging from 20.6-135 μg kg^{-1}. Fumonisins were detected in all the corn samples (100%) ranging from 261-2.420 μg kg^{-1}. Although, only 80 samples were analyzed, they were randomly collected from 10 states in Malaysia. Since there is a lack of information from Malaysia and such data are valuable.

Key words: Corn, moulds, mycotoxins, animal feed, ELISA

INTRODUCTION

Corn is the world's third most important crop after rice and wheat. About half of this is grown in developing countries where corn flour is a staple food for poor people and corn stalks provide dry season feed for farm animals (Desjardins and Busman, 2006; Roige *et al*., 2009). In industrialized countries, corn is largely used as livestock feed and as raw material for industrial products e.g., in Malaysia as feed, silage, breakfast food and processing (breakfast cereals, corn chips, grits and flour), industrial starch and popcorn. Corn is an important feed ingredient for the livestock industry in Malaysia (Warr *et al*., 2008). It is not a staple food crop in Malaysia and is totally imported from Argentina, China, Indonesia and Thailand. Corn imports have grown rapidly to US$398 million in 2006 in response to stronger demand from the livestock industry (Warr *et al*., 2008).

Corn is also one of the crops subject to the most critical mycotoxin problems throughout the world. Mycotoxin production may occur in the field during post harvest, storage, processing or feeding under appropriate environmental conditions. These metabolites are generally associated with fungi belonging to the genera *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium* (Osweiler, 2000; Roige *et al*., 2009; Reddy *et al*., 2010). Toxigenic *Alternaria* and *Fusarium* species are often classified as field fungi while *Aspergillus* and *Penicillium* species are considered storage fungi (Logrieco *et al*., 2003; Niaz and Dawar, 2009). The major classes of mycotoxins of concern in feedstuffs include aflatoxins, Deoxynivalenol (DON) and other trichothecenes, Zearalenone (ZEN), fumonisins, Ochratoxin A (OTA) and ergot alkaloids (Driehuis *et al*., 2008). Of these mycotoxins, DON has the greatest prevalence in feedstuffs and is found in forage feeds in forage corn in particular and in ingredients for concentrate feeds (Whitlow and Hagler, 2005; Driehuis *et al*., 2008).

Several researchers have been documented mycotoxins in corn based feedstuffs. For example, Tangendjaja *et al*. (2008) detected several mycotoxins in corn used by feed mills in Indonesia. In another study, Bahri found only aflatoxins in corn feed. Yoshizawa *et al*. (1996) found natural occurrence of aflatoxins and fumonisins in corn in Thailand. Recently, Roige *et al*. (2009) detected aflatoxins, DON and ZEA in corn samples used for pig and cattle feeds in Argentina. However, mycotoxin reports from Malaysia are scarce. Only recently aflatoxins has been reported in wheat, barley, peanuts, nutty products and feed while DON have been recovered from wheat based noodles (Sulaiman *et al*., 2007; Leong *et al*., 2009; Moazami and Jinap, 2009; Khayoon *et al*., 2010; Reddy and Salleh, 2010).

A report by the Food and Agriculture Organization (FAO) of the United Nations (FAO, 2004) on mycotoxin regulations around the world revealed that at least 77 countries now have specific regulations for acceptable concentrations of mycotoxins in foods and feeds (Van Egmond *et al*., 2007). However, there are no

Corresponding Author: K.R.N. Reddy, School of Biological Sciences, Universiti Sains Malaysia, 11800 USM, Penang, Malaysia

mycotoxin regulations for Malaysian feeds till today. The fact that countries have no specific regulatory limit for mycotoxins does not mean that the problem is ignored. Several countries have recognized that they have problems due to mycotoxins and that regulations should be adopted (Zainidine and Manes, 2009). Mycotoxin profiles in corn and its products from Malaysia are necessary as it is being used as huge quantities of feed in poultry farms. Thus, the aim of this study was to determine the mycoflora and mycotoxins in corn grains intended to use for animal feed in Malaysia.

MATERIALS AND METHODS

Collection of corn grain samples and isolation of moulds: A total of 80 corn grain samples intended to use as animal feed were collected from 10 states in Malaysia during December 2009 to August 2010 (Table 1) and analyzed for fungal mycoflora using the agar-plate method according to Reddy *et al.* (2009) and Lee *et al.* (2009). About 100 corn grains from each sample were placed on one-half strength potato dextrose agar medium containing rose bengal at a concentration of 50 mg L^{-1} for *Aspergillus* sp. and on peptone-PCNB for *Fusarium* sp. and on potato dextrose agar for other fungal mycoflora. The plates were incubated at 25±2°C for 6 days and then the fungal colonies were counted. The fungal species of each colony were identified according to the methods by Klich (2002) for *Aspergillus* sp., Leslie and Summerell (2006) for *Fusarium* sp. and other fungi by Dugan (2006).

Materials: Commercial ELISA kits for AFB1 (5121AFB1p) and fumonisins (Agraquant® COQAK 3000) were obtained from Euproxima (The Netherlands) and Romer Labs Pvt. Ltd. (Singapore). All other solvents and chemicals were obtained from Merck, Darmstadt, Germany.

Sample preparation for mycotoxins analysis: Samples preparation and test method were conducted according to manufacturer's instructions. Briefly, all corn grain samples were ground to fine powder (<200 μm) and sub-samples of 20 g were mixed with 60 mL of 80% methanol for AFB1 and 100 mL of 70% methanol for fumonisins and shaken in a waring blender at high speed for 3 min. The extract was allowed to settle and then filtered through a Whatman No 1 (Whatman International Ltd., England) filter paper. The filtrate was diluted to 1:6 and 1:20 with distilled water for ELISA analysis.

Determination of AFB1 by ELISA: According to manufacturer's instructions, standard solutions and prepared samples (50 μL) were added to microtiter wells in duplicate followed by 25 μL of conjugate (Aflatoxin-HRP) and 25 μL of antibody (anti AFB1). After 60 min incubation at 37°C, the wells were washed 3 times with 300 μL of rinsing buffer. Substrate (100 μL) was added to each well and incubated at room temperature for 30 min. Following the addition of stop solution (100 μL) to each well, the intensity of the resulting yellow color was measured at a wavelength of 450 nm using 96 well plate reader (Robonik®, India). The detection and quantification limits were 0.5 and 0.75 μg kg^{-1}, respectively.

Determination of fumonisins by ELISA: According to manufacturer's instructions, a sufficient number of microtiter wells were inserted into the microwell holder for standards and samples to be run in duplicate. Briefly, standard solutions and prepared samples (100 μL) were mixed with 200 μL of conjugate in individual dilution wells and then 100 μL from each dilution well was transferred to respective antibody-coated well. After 10 min incubation at room temperature wells were washed 5 times with 250 μL distilled water. Substrate (100 μL) was added to each well and incubated at room temperature for 5 min. Following the addition of stop solution (100 μL) to each well, the intensity of the resulting yellow color was measured at a wavelength of 450 nm using 96 well plate reader (Robonik®, India). The detection and quantification limits were 200 and 250 μg kg^{-1}, respectively.

Calculation of mycotoxin concentration: The absorbance values obtained for standards and the samples were divided by the absorbance value of the first standard (zero standard) and multiplied by 100 (percentage of maximal absorbance). The absorption intensity was found to inversely proportional to mycotoxin concentration in the samples. The log-logit sheets supplied with the kits were used to generate a standard curve and to calculate the concentration of both toxins in each sample.

Method validation: Validation of ELISA method was carried out by determination of recoveries of uncontaminated samples spiked at 2.5 and 5 μg kg^{-1} for AFB1 and 250 and 500 μg kg^{-1} for fumonisins. The Repeatability (RSDr) and Reproducibility (RDS_R) were also calculated at spiking levels as mentioned above. The repeatability was estimated under repetitive conditions at the same day while reproducibility was estimated at time intervals (at 6 different days of the month).

RESULTS

Co-occurrence of moulds in corn grain samples: Co-occurrence of fungal infection in corn samples are shown in Table 1. *Aspergilus* and *Fusarium* sp. are the frequent fungal contaminants in all corn samples.

Table 1: Mycoflora content of corn samples from Malaysia

Place of sample collection	Samples collected	No. of colonies detected (% infection)						
		A. fl	*A. ni*	*F. ve*	*F. gr*	*F. pr*	*F. eq*	*Pen*
Johor	8	800 (100)	800 (100)	352 (44)	452 (56)	365 (46)	258 (32)	0 (0.0)
Kedah	6	600 (100)	600 (100)	454 (76)	258 (43)	258 (43)	205 (34)	102 (17.0)
Kelatan	8	800 (100)	652 (81)	422 (53)	365 (46)	256 (32)	365 (46)	45 (6.0)
Melaka	10	627 (63)	661 (66)	321 (32)	150 (15)	562 (56)	121 (12)	20 (2.0)
Pahang	7	700 (100)	464 (66)	362 (52)	452 (64)	452 (64)	212 (30)	0 (0.0)
Pedas	4	400 (100)	400 (100)	255 (64)	125 (31)	125 (31)	106 (26)	0 (0.0)
Penang	15	1,500 (100)	1,500 (100)	698 (47)	856 (57)	458 (30)	365 (24)	125 (8.3)
Perlis	7	456 (65)	373 (53)	245 (35)	356 (51)	325 (46)	253 (36)	25 (3.6)
Perak	8	405 (51)	494 (62)	264 (33)	258 (32)	312 (39)	362 (45)	60 (7.5)
Selangor	7	696 (99)	664 (95)	358 (51)	189 (27)	236 (34)	165 (23)	22 (3.1)
Total	80	6,984 (87)	6,608 (83)	3,731 (47)	3,461 (43)	3,349 (42)	2,412 (30)	399 (5.0)

A. fl = *A. flavus*, *A. ni* = *A. niger*, *F. ve* = *F. verticillioides*, *F. gr* = *F. graminearum*, *F. pr* = *F. proliferatum*, *F. ep* = *F. equisieti*, *Pen* = *Penicillium* sp.

Aspergillus flavus (87%) and *A. niger* (83%) were the most frequently found species being present in all samples followed by *F. verticillioides* (47%), *F. graminearum* (43%), *F. proliferatum* (42%), *F. equisieti* (30%) and *Penicillium* sp. (5%) (Table 1). In some samples, *Mucor* and *Rhizopus* species overgrew all other fungi and inhibited colony development of others. A total of 409 fungal isolates including *Aspergillus* sp. (160), *Fusarium* sp. (240) and *Penicillium* sp. (9) were recovered and purified using the single spore technique and stored in the culture collection centre at Universiti Sains Malaysia for further studies. The occurrence of *A. flavus* (100%) was high in the samples collected from Johor, Kedah, Kelatan, Pahang, Pedas and Penang whereas *A. niger* (100%) in samples from Johor, Kedah, Kelatan, Pedas and Penang (Table 1). Highest occurrence of *F. verticillioides* (76%), *F. graminearum* (64%), *F. proliferatum* (64%), *F. equisieti* (46%) and *Penicillium* sp. (17%) was observed in the samples collected from Kedah, Pahang, Pahang, Kelatan and Kedah, respectively (Table 1).

Co-occurrence of mycotoxins in corn grain samples: The ranges with the number of samples positive for each mycotoxin are shown in Table 2. Aflatoxin B_1 (AFB1) could be detected in 65 (81.2%) corn samples ranging from 1.0-135.1 μg kg^{-1}. About 18 (22.5%) samples, out of 80 had exceeded AFB1 above the international regulatory limits of animal feeds (>20 μg kg^{-1}) ranging from 20.6-135 μg kg^{-1}.

The highest AFB1 levels were detected in samples collected from Penang (up to 135 μg kg^{-1}) and Melaka (up to 120 μg kg^{-1}) (Table 2). Fumonisin levels detected in all corn samples (100%) were below the internationally permissible limits (<5.000 μg kg^{-1}) fixed for corn based animal feed ranging from 261-2.420 μg kg^{-1}. The highest fumonisin levels were detected in samples collected from Melaka (2.420 μg kg^{-1}) followed by Penang (2.370 μg kg^{-1}).

Table 2: Mycotoxins detected in corn samples

Place of sample collection	AFB1		Fumonisins	
	Positive samples	Range (μg kg^{-1})	Positive samples	Range (μg kg^{-1})
Johor	6	3.0-29	8	280-690
Kedah	6	1.0-19	6	310-350
Kelatan	7	2.0-16	8	340-895
Melaka	8	5.0-120	10	270-2,420
Pahang	5	16-19	7	331-505
Pedas	4	2.0-33	4	294-580
Penang	14	3.0-135	15	280-2,370
Perlis	6	6.0-32	7	359-860
Perak	5	1.0-6.0	8	440-615
Selangor	4	3.0-49	7	261-288
Total	65	1.0-135	80	261-2,420

Table 3: Recoveries of mycotoxins from spiked samples

Mycotoxin spiked	Recovered (μg kg^{-1})	Percentage recovery	Repeatability (RSDr, %)	Reproducibility (RDS_R, %)
AFB1 (2.5 μg kg^{-1})	2.25±0.91	90.0±2.65	1.4	6.2
AFB1 (5.0 μg kg^{-1})	4.65±0.22	93.0±1.26	1.9	4.2
Fumonisins (250 μg kg^{-1})	235.6±2.65	94.2±3.65	2.6	8.4
Fumonisins (500 μg kg^{-1})	488.6±1.12	97.7±1.25	2.1	9.5

Values are average of three replications with standard deviation

Recoveries of mycotoxins from spiked samples: The average recoveries with SDs were 90.0±2.65% to 93.0±1.26% for AFB1 and 94.2±3.65 and 97.7±1.25% for fumonisins, respectively. The repeatability and reproducibility were in the range of 1.4-2.6 and 4.2-9.5% for both mycotoxins tested (Table 3).

DISCUSSION

Mycotoxigenic fungi belonging to Aspergillus, Fusarium and Penicillium are the most frequently isolated from corn grains throughout the world (Ghiasian *et al*., 2006; Roige *et al*., 2009). *Aspergillus* and *Fusarium* species are also known to cause diseases in corn called Aspergillus ear rot and Fusarium ear rot, respectively (Naidoo *et al*., 2002; Logrieco *et al*., 2007).

Recently, Roige *et al.* (2009) reported that *Penicillium* (70%), *Fusarium* (47%) and *Aspergillus* (34%) were the most frequent and abundant genera in corn grain samples used for cattle and pig feed in Argentina. In another study, *Fusarium* and *Penicillium* genera were the most prevalent taxa of the internal seed-borne mycobiota in corn seed samples obtained from five different production regions in Argentina while *Aspergillus* genera was found at low frequency (Gonzalez *et al.*, 2001). Domijan *et al.* (2005) reported most of the maize grain samples were contaminated with *Fusarium* and *Penicillium* sp. and few samples contaminated with *Aspergillus* sp. in Croatia. Tapia *et al.* (2005) reported that *Penicillium* sp. was the most prevalent species in corn based feed. In the present study, *Aspergillus* sp. was the dominant fungi in all corn samples followed by *Fusarium* and *Penicillium* sp. Differences in geographical and environmental conditions might be responsible for the differences in fungal distribution. However, the results are in agreement with that previous report indicating that Aspergillus was the most prevalent moulds isolated from feed in Europe (Krustev and Kristov, 1981).

The results showed that *A. flavus* (87%) was dominant in all most all the corn grain samples followed by *A. niger* (83%). These results are in agreement with the previous findings by Trung *et al.* (2008) who had reported *A. flavus* as the most frequent contaminant in 90% of their maize samples in Vietnam. Similarly, Niaz and Dawar (2009) found 70% of their maize samples from Pakistan were contaminated with *Aspergillus* and *Penicillium* sp. Gonzalez *et al.* (2008) also reported *Aspergillus* sp. was the most dominant genera in corn samples in Argentina. In another study, Purwoko *et al.* (1991) observed *A. flavus* and *A. parasiticus* were the most dominant fungi in corn feed from Indonesia. Similarly, Dutta and Das (2001) isolated aflatoxigenic *A. flavus* and *A. parasiticus* in feed derived from corn in India. Desjardins and Busman (2006) stated that maize is frequently contaminated with *Fusarium* sp. mainly *F. verticillioides* and *F. proliferatum* which produces fumonisins and *F. graminearum* which produces trichothecenes mainly nivalenol and DON in Nepal. Recently, Dorn *et al.* (2009) recovered 16 different *Fusarium* sp. from kernels and 15 species from stem pieces in Switzerland. In this study, we observed the above said three *Fusarium* sp. in all the corn samples. Apart from these we also recovered *F. equisieti* which is responsible for production of ZEN in all corn samples.

Several researchers frequently detected aflatoxins, fumonisins, DON, ZEN and trichothecenes in corn grains and its products throughout the world (Pietri *et al.*, 2009; Dorn *et al.*, 2009; Khayoon *et al.*, 2010). In the study, 80 corn grain samples used for animal feed collected from various states in Malaysia were evaluated for AFB1 and fumonisins contamination. More recently, Khayoon *et al.* (2010) detected only aflatoxins in various animal feeds in Malaysia. They found total aflatoxins in corn (8.6 μg kg^{-1}) and corn germ meal (45.6 μg kg^{-1}). In the study, AFB1 was detected in 65 (81.2%) samples ranging from 1.0-135 μg kg^{-1}. About 18 (22.5%) samples had exceeded AFB1 levels above the legal limits of many countries (>20 μg kg^{-1}) whereas fumonisins in all corn samples were below the regulatory limits.

Surveys from other countries have reported the occurrence of aflatoxins in corn and related products from Brazil (Sekiyama *et al.*, 2005), China (Li *et al.*, 2001), India (Shetty and Bhat, 1997), Italy (Pietri *et al.*, 2009), Nigeria (Bankole and Mabekoje, 2004; Manjula *et al.*, 2009), Turkey (Castells *et al.*, 2008) and USA (Rosen and Rosen, 1984) and the contamination of animal feeds has been reported in Greece, Turkey (Aycicek *et al.*, 2005).

The results showed that presence of fumonisins in all corn samples ranging from 261-2.420 μg kg^{-1}. These results are in agreement with the previous findings of Nuryono *et al.* (2002) who had reported fumonisins in 16 corn based feed samples out of 17 ranging from 17.6-3.306 μg kg^{-1} in Indonesia. Gonzalez *et al.* (2008) reported FB1 and FB2 in all feed and corn samples used for pig feed in central Argentina whereas all samples were negative for aflatoxins and ZEN.

Recently, Dorn *et al.* (2009) found high concentrations of fumonisins in all maize kernels ranging from 2.5-22.8 mg kg^{-1} in 2005 and from 9.9-28.6 mg kg^{-1} in 2006. Charoenpornsook and Kavisarasai (2006) detected AFB1 in 23/25 samples (average of 7.56 μg kg^{-1}), OTA in 3/10 samples (10.48-12.35 μg kg^{-1}), DON in 13/15 samples (33.77 μg kg^{-1}) and T-2 toxin in all 10 samples (6.91 μg kg^{-1}) of animal feedstuffs in Thailand. Samples of maize (118) imported into Taiwan were detected for FB1 and found 8 (6.8%) samples contain FB1 ranging from 334-1.614 μg kg^{-1} (Tseng and Liu, 2001). However, further studies are warranted to analyze mycotoxins in large number of feed samples to develop suitable management strategies.

CONCLUSION

The results showed that corn grain samples intended to use for animal feed in Malaysia were contaminated with moulds and mycotoxins. More than 50% of corn samples were found contaminated with mycotoxins. About 18 (22.5%) samples, out of 80 had exceeded the AFB1 above the legal limits of Argentina, Australia, Brazil, India, Netherlands and USA (>20 μg kg^{-1}). Fumonisin levels in all corn samples were below the international acceptable limits in animal feeds. However, the detection

of small quantities in all the corn samples warrants further investigations as the total usage of corn derived feed in livestock industry is very high in Malaysia. A possible reason for the occurrence of moulds and mycotoxins is the inappropriate storage during production. Based on the results of this study, we can expect that there is a mycotoxin problem for livestock industry in Malaysia. However, livestock industries in Malaysia should develop mycotoxin detection methods by themselves or they should send samples to mycotoxin detection laboratories to confirm that their feedstuffs are negative for mycotoxins.

We hope this study will facilitate towards the development of regulatory limits and proper storage structures and suitable management practices in the country.

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REFERENCES

Aycicek, H., A. Aksoy and S. Saygi, 2005. Determination of aflatoxin levels in some dairy and food products consumed in Ankara, Turkey. Food Control, 16: 263-266.

Bankole, S.A. and O.O. Mabekoje, 2004. Occurrence of aflatoxins and fumonisins in preharvest maize from Southwestern Nigeria. Food Addit. Contam., 21: 251-255.

Castells, M., S. Marin, V. Scanchis and A.J. Ramos, 2008. Distribution of fumonisins and aflatoxins in corn fractions during industrial cornflake processing. Int. J. Food Microbiol., 123: 81-87.

Charoenpornsook, K. and P. Kavisarasai, 2006. Mycotoxins in animal feedstuffs of Thailand. KMITL Sci. Technol. J., 6: 25-28.

Desjardins, A.E. and M. Busman, 2006. Mycotoxins in developing countries: A case study of maize in Nepal. Mycotoxin Res., 22: 92-95.

Domijan, A.M., M. Peraica, B. Cvjetkovic, S. Turin, E. Jurjevic and D. Ivic, 2005. Mould contamination and co-occurrence of mycotoxins in maize grain in Croatia. Acta Pharma, 55: 349-356.

Dorn, B., H.R. Forrer, S. Schurch and S. Vogelgsang, 2009. *Fusarium* species complex on maize in Switzerland: Occurrence, prevalence, impact and mycotoxins in commercial hybrids under natural infection. Eur. J. Plant Pathol., 125: 51-61.

Driehuis, F., M.C. Spanjer, J.M. Scholten and M.C. Te-Giffel, 2008. Occurrence of mycotoxins in feedstuffs of dairy cows and estimation of total dietary intakes. J. Dairy Sci., 91: 4261-4271.

Dugan, F.M., 2006. The Identification of Fungi: An Illustrated Introduction with Keys, Glossary and Guide to Literature. APS Press, St Paul, MN USA., pp: 176.

Dutta, T.K. and P. Das, 2001. Isolation of aflatoxigenic strain of *Aspergillus* and detection of aflatoxin B-1 from feed in India. Mycopathologia, 151: 29-33.

FAO, 2004. Worldwide Regulations for Mycotoxins in Food and Feed in 2003. Food and Agriculture Organization, Rome, pp: 1-180.

Ghiasian, S.A., A.H. Maghsood, H. Yazdanpanah, G.S. Shephard and L. van der Westhuizen *et al*., 2006. Incidence of *Fusarium verticillioides* and levels of fumonisins in corn from main production areas in Iran. J. Agric. Food Chem., 54: 6118-6122.

Gonzalez, H.H.L., S.L. Resnik and A.M. Pacin, 2001. Myoflora of freshly harvested flint corn from Northwestern Provinces in Argentina. Mycopathologia, 155: 207-211.

Gonzalez, P.M.L., C.M. Pereyra, M.L. Ramirez, C.A.R. Rosa, A.M. Dalcero and L.R. Cavaglieri, 2008. Determination of mycobiota and mycotoxins in pig feed in central Argentina. Lett. Applied Microbiol., 46: 555-561.

Khayoon, W.S., B. Saad, C.B. Yan, N.H. Hashim, A.S.M. Ali, M.I. Salleh and B. Salleh, 2010. Determination of aflatoxins in animal feeds by HPLC with multifunctional column clean-up. Food Chem., 118: 882-886.

Klich, M.A., 2002. Identification of Common *Aspergillus* Species. Centreal Bureau Voor Schimmel Culture, AD Utrecht, Netherland, pp: 116.

Krustev, E. and B. Kristov, 1981. The mycoflora in maize silage. Veterinary Nauki, 18: 88-91.

Lee, J., I.Y. Chang, H. Kim, S.H. Yun, J.F. Leslie and Y.W. Lee, 2009. Genetic diversity and fitness of *Fusarium graminearum* populations from rice in Korea. Applied Environ. Microbiol., 75: 3289-3295.

Leong, Y.H., N. Ismail, A.A. Latif and R. Ahmad, 2009. Aflatoxin occurrence in nuts and commercial nutty products in Malaysia. Food Control, 21: 334-338.

Leslie, J.F. and B.A. Summerell, 2006. The *Fusarium* Laboratory Manual. 1st Edn., Blackwell Publishing, Iowa, USA., pp: 388.

Li F.Q., T. Yoshizawa, O. Kawamura, X.Y. Luo and Y.W. Li, 2001. Aflatoxins and fumonisins in corn from the high-incidence area for human hepatocellular carcinoma in Guangxi, China. J. Agric. Food Chem., 49: 4122-4126.

Logrieco, A., A. Bottalico, G. Mule, A. Moretti and G. Perrone, 2003. Epidemiology of toxigenic fungi and their associated mycotoxins for some Mediterranean crops. Eur. J. Plant Pathol., 109: 645-667.

Logrieco, A., A. Moretti, G. Perrone and G. Mule, 2007. Biodiversity of complexes of mycotoxigenic fungal species associated with *Fusarium* ear rot of maize and *Aspergillus* rot of grape. Int. J. Food Microbiol., 119: 11-16.

Manjula, K., K. Hell, P. Fandohan, A. Abass and R. Bandyopadhyay, 2009. Aflatoxin and fumonisin contamination of cassava products and maize grain from markets in Tanzania and republic of the Congo. Toxin Rev., 28: 63-69.

Moazami, E.F. and S. Jinap, 2009. Natural occurrence of deoxynivalenol (DON) in wheat based noodles consumed in Malaysia. Microchem. J., 93: 25-28.

Naidoo, G., A.M. Forbes, C. Paul, D.G. White and T.R. Rocheford, 2002. Resistance to *Aspergillus* ear rot and aflatoxin accumulation in maize F1 hybrids. Crop Sci., 42: 360-364.

Niaz, I. and S. Dawar, 2009. Detection of seed borne mycoflora in maize (*Zea mays* L.). Pak. J. Bot., 41: 443-451.

Nuryono, A., C.T. Noviandi, A. Agus, S. Wedhastri, Y.B. Maryudani, J. Bohm and E. Razzazi, 2002. A survey of fumonisins (B1, B2, B3) in Indonesian corn-based food and feed samples. Mycotoxin Res., 18: 117-120.

Osweiler, G.D., 2000. Mycotoxins: Contemporary issues of food animal health and productivity. Vet. Clin. North Am. Food Anim. Pract., 16: 511-530.

Pietri, A., M. Zanetti and T. Bertuzzi, 2009. Distribution of aflatoxins and fumonisins in dry-milled maize fractions. Food Additives Contaminants, 26: 372-380.

Purwoko, H.M., B. Hald and J. Wolstrup, 1991. Aflatoxin content and number of fungi in poultry feedstuffs from Indonesia. Lett. Applied Microbiol., 12: 212-215.

Reddy, K.R.N., B. Salleh, B. Saad, H.K. Abbas, C.A. Abel and W.T. Shier, 2010. An overview of mycotoxin contamination in foods and its implications for human health. Toxin Rev., 29: 3-26.

Reddy, K.R.N., C.S. Reddy and K. Muralidharan, 2009. Detection of *Aspergillus* spp. and aflatoxin B1 in rice in India. Food Microbiol., 26: 27-31.

Reddy, K.R.N., and B. Salleh, 2010. A preliminary study on the occurrence of *Aspergillus* spp and aflatoxin B1 in imported wheat and barley in Penang, Malaysia. Mycotoxin Res., 26: 267-271.

Roige, M.B., S.M. Aranguren, M.B. Riccio, S. Pereyra, A.L. Soraci and M.O. Tapia, 2009. Mycobiota and mycotoxins in fermented feed, wheat grains and corn grains in Southeastern Buenos Aires Province, Argentina. Rev. Iberoam Mycol., 26: 233-237.

Rosen, R.T. and J.D. Rosen, 1984. Quantification and confirmarion of four *Fusarium mycotoxins* in corn by gas chromatography-mass spectrometry-selected ion monitoring. J. Chromatography A, 283: 223-230.

Sekiyama, B.L., A.B. Ribeiro, P.A. Machinski and M.M. Junior, 2005. Aflatoxins, ochratoxin A and zearalenone in maize-based food products. Brazilian J. Microbiol., 36: 289-294.

Shetty, P.H. and R.V. Bhat, 1997. Natural occurrence of fumonisin B1 and its co-occurrence with aflatoxin B1 in Indian sorghum, maize, and poultry feeds. J. Agric. Food Chem., 45: 2170-2173.

Sulaiman, M.R., C.F. Yee, A. Hamid and A.M. Yatim, 2007. The occurrence of aflatoxins in raw shelled peanuts samples from three districts of Perak, Malaysia. Electronic J. Environ. Agric. Food Chem., 6: 2045-2052.

Tangendjaja, B., S. Rachmawati and E. Wina, 2008. Mycotoxin contamination on corn used by feed mills in Indonesia. Indonesian J. Agric. Sci., 9: 68-76.

Tapia, M.O., M.D. Stern, A.L. Soraci, R. Meronuck and W. Olson *et al*., 2005. Patulin-producing molds in corn silage and high moisture corn and effects of patulin on fermentation by ruminal microbes in continous culture. Anim. Feed Sci. Technol., 119: 247-258.

Trung, T.S., C. Tabuc, S. Bailly, A. Querin, P. Guerre and J.D. Bailly, 2008. Fungal mycoflora and contamination of maize from Vietnam with aflatoxin B1 and fumonisin B1. World Mycotoxin J., 1: 87-94.

Tseng, T.C. and C.Y. Liu, 2001. Occurrence of fumonisin B1 in maize imported into Taiwan. Int. J. Food Microbiol., 65: 23-26.

Van Egmond, H.P., R.C. Schothorst and M.A. Jonker, 2007. Regulations relating to mycotoxins in food perspectives in a global and European context. Anal. Bioanal. Chem., 389: 147-157.

Warr, S., G. Rodriguez and J. Penm, 2008. Changing food consumption and imports in Malaysia. Research Report 08.6, pp: 1-29. http://www.abare.gov.au/publications_html/crops/crops_08/malaysiafood.pdf.

Whitlow, L.W. and W.M. Hagler, 2005. Mycotoxins: A review of dairy concerns. Proceedings of the Mid-South Ruminant Nutrition Conference (MARNC'05), Texas Animal Nutrition Council, Dallas, TX USA., pp: 47-58.

Yoshizawa, T., A. Yamashita and N. Chokethaworn, 1996. Occurrence of fumonisins and aflatoxins in corn from Thailand. Food Additives Contam., 13: 163-168.

Zainidine, A. and J. Manes, 2009. Occurrence and legislation of mycotoxins in food and feed from Morocco. Food Control, 20: 334-344.