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ASPERGILLUS FLAVUS INFECTION AND AFLATOXIN CONTAMINATION IN PEANUTS AT VARIOUS STAGES OF THE DELIVERY CHAINS IN WONOGIRI REGENCY, CENTRAL JAVA, INDONESIA

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ABSTRACT

As a part of an Australian Centre for International Agricultural Research (ACIAR) project on management of aflatoxin in Indonesia and Australia (PHT 97/017), a survey on pre- and postharvest handling of peanuts at farmer, collector retailer levels, including *Aspergillus flavus* infection and aflatoxin B1 contamination of peanuts collected in Wonogiri regency, Central Java, was conducted during the harvest period of the wet (February 2003) and dry (June 2003) seasons. Interviews using questionnaires, with farmers, collectors and retailers concerning pre- and post-harvest handling of peanuts were carried out. The moisture contents and physical quality of the peanut kernels were also determined. During the wet and dry seasons, fresh pod samples were collected from farmer fields (24 samples), dry raw pod samples from collector (24 samples) and raw kernel samples from retailer levels (54 samples). Thus, during each season 102 samples of various kinds of peanuts were collected for analysis. The *A. flavus* infection in kernel samples of peanuts collected from retailers were the highest, followed by samples collected from collectors and farmers. The *A. flavus* infection in kernel samples collected during the dry season was in general lower than in the wet season. It was alarming to note that all most all samples of raw kernel samples collected from retailers were infected by *A. flavus* during the wet and dry seasons. In general aflatoxin B1 levels in peanut samples collected in the wet season were higher than the dry season. During the wet and dry seasons, the aflatoxin B1 contents in retailer samples were the highest, followed by those collected from collectors and farmers. During the wet season, around 4, 17 and 33% of peanut samples collected from farmers, collectors and retailers, were respectively contaminated with more than 15 ppb of aflatoxin B1. During the dry season, around 42 and 74% of peanut samples collected from collectors and retailers, were respectively contaminated with more than 15 ppb of aflatoxin B1, while aflatoxin levels in wet raw pod samples collected from farmers were less than 15 ppb. In 2003 Codex Alimentarius Commission has determined a maximum level of total aflatoxins in peanuts intended for further processing at 15 ppb.

Key words: *Aspergillus flavus* / aflatoxin / peanuts/Wonogiri regency

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INTRODUCTION

Aflatoxin is a human and domestic animal carcinogen that can contaminate peanuts and hence is a major food quality problem throughout the world, but is particularly severe in developing countries such as Indonesia. It occurs when kernels become infected by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*, under drought stress before harvest, during the drying phase in the field, or under poor storage conditions.

Pitt and Hocking (1996) reported that 45% of 215 peanut samples collected from farm storage, middlemen and retailers in Bogor (West Java), Yogyakarta (Yogyakarta Special Territory), and their surroundings, contained more than 50 ppb of aflatoxin, 33% more than 300 ppb, and 22 % exceeded 1,000 ppb.

According to Dharmaputra *et al.* (2003b), in general aflatoxin B1 contents of peanuts collected from farmer's fields/*penebas*/collectors and processed samples in the Pati regency of Central Java were low (less than 15 ppb). The highest aflatoxin B1 contents were found in raw peanut kernels collected from retailers in traditional markets, ranging from 2 – 124 and < 4 - 342 ppb during the wet and dry seasons in 2002, respectively.

The percentage of raw kernel samples contaminated with aflatoxin B1 (exceeding 15 ppb) collected during the wet and dry seasons was 33 and 25%, respectively.
In 2003 Codex Alimentarius Commission has determined a maximum level of total aflatoxins in peanuts intended for further processing at 15 ppb. In Australia the maximum allowable limit of aflatoxin in peanut and peanut products is 15 ppb (QDPI 2000). On 9 September 2004 the National Agency for Drug and Food Control, Republic of Indonesia has determined that aflatoxin B1 and total aflatoxin contents in processed peanut products should not be more than 20 and 35 ppb, respectively.
To minimize or to reduce aflatoxin contamination in peanuts, appropriate post-harvest handling methods in each level of peanut delivery chain (farmer, collector, and retailer) should be carried out.

The objective of this study was to obtain information on pre- and postharvest handling methods, *Aspergillus flavus* infection and aflatoxin B1 contamination of peanuts collected from different points of the delivery chains in Wonogiri regency of Central Java. The moisture contents and physical quality of peanut kernels were also determined.

MATERIALS AND METHODS

Time and location of surveys

Surveys were conducted during the wet (February 2003) and dry (June 2003) seasons in Wonogiri regency, Central Java. The regency was selected because it produces high quantities of peanuts.

The study comprised the following activities:

- Interviews using questionnaires with farmers, collectors and retailers aimed at collecting information on pre- and post-harvest handling of peanuts.
- Sampling peanuts at various points in the peanut supply chain (i.e farmers, collectors and retailers) for aflatoxin analysis. Some of peanut samples were also collected from retailers in traditional markets in the city of Surakarta (approximately 35 km from Wonogiri).

Sampling methods

At farmer level, peanut samples are collected from a peanut farm. About 20 peanut plants were selected randomly; and pods were hand-harvested manually to obtain about 2 kg of wet raw pod peanuts. At collector level, three samples (=three replications) of dry raw pod peanuts (about 2 kg/sample) were collected randomly from each collector; the peanuts have been placed in woven polypropylene bags. At retailer level, samples of raw kernels (1 kg/sample) were collected from each retailer in traditional market.

The 2 kg samples of wet and dry raw pods collected from collectors and 1 kg of raw kernel samples collected from traditional market were sub-divided in to three replications to record information on moisture content, physical quality of kernels, percentage of kernels infected by *A. flavus*, and aflatoxin B1 content. Wet and dry raw pods were then shelled manually.

Moisture content, physical quality of kernels, *A. flavus* and aflatoxin B1 analyses

Moisture contents of kernels (based on wet basis) derived from various kinds of peanuts were analyzed using oven method (BSI 1995). Two replicates were used from each sample. Physical quality of kernels included visual assessment of intact, shriveled and damaged kernels. The damaged kernels included cracked, broken, discoloured, and damage caused by insects or fungi. The percentage of each category of kernels was determined by counting them and dividing the total number of kernels used for physical quality analysis. The kernels infected by *A. flavus* was determined using 100 kernels per sample and plating method with *Aspergillus flavus* and *parasiticus agar* (AFPA) (Pitt *et al.* 1983). Aflatoxin B1 was analyzed using the ELISA method (Lee and Kennedy 2002), with two replicates used for each sample.

RESULTS AND DISCUSSION

Source, kind and number of samples

During the wet and dry seasons, fresh and dry pod samples were collected from farmer fields (24 samples) and collector levels (24 samples), respectively. Twenty-seven samples of raw kernels were collected from traditional markets in Wonogiri regency and the city of Surakarta, respectively. Thus, during each season 102 samples of various

kinds of peanuts were collected. Details of the peanut delivery chain, location, kind and number of peanut samples collected during the wet and dry seasons are presented in Table 1.

Results of interviews with farmers, collectors and retailers concerning pre- and post-harvest handling of peanuts

Interview with farmers

Most of the peanut growing environments was dry land conditions, with most farmers having mixed cropping with maize or cassava. During planting all of the respondents used fertilizer, controlled the weeds manually, and did not apply insecticides. Thirty-three and 50% of the respondents interviewed during the wet and dry seasons, respectively, sold their peanuts to collectors in the form of wet raw pods after harvest, while other respondents sold peanuts to retailers in traditional markets in the form of wet raw pods (29%) and dry kernels (13%) during the wet season. Farmer's sun-dried peanut pods on bamboo mat or paved floor, nevertheless, most of them used bamboo mat. All respondents were not aware of aflatoxin problem in peanuts.

Interview with collectors

At the collectors peanuts were sun-dried on bamboo mats (71% of respondents during the wet season) and on woven paved floors (88% of respondents during the dry season). Some of collectors sold peanuts directly to peanut factories in Pati, Central Java, in the form of wet raw pods. Collectors stored dried peanut pods in polypropylene bags for 7 days up to 6 months before selling them to retailers in traditional markets in the districts of Wonogiri regency and in the city of Surakarta in the form of dry kernels and to retailers in traditional markets of big cities in Central Java in the form of dry raw pods. Most of the collectors did not monitor the physical quality of peanuts before selling to retailers or peanut factories in Pati.

Interview with retailers

Most of the retailers at traditional markets in Wonogiri regency and the city of Surakarta bought peanuts from farmers, while some of them bought peanuts from collectors. During the wet and dry seasons, 33 and 47% of the respondents, respectively, stored peanuts in jute bags, while 33 and 32% of the respondents, respectively, stored peanuts in recyclable (plastic) bags for less than one week. Apart from peanuts, retailers sold other commodities such as milled rice and other secondary crops (maize, soybean, mungbean), garlic, onion, sugarpalm, *kerupuk*, *emping* and cashew. Peanut buyers were also sellers of processed peanut products including *gado-gado, pecel* and *sate* sauce. All of respondents were also not aware of aflatoxin problem in peanuts.

Table 1. Location of sampling, kind and number of peanut samples collected from different points of the delivery chains during the wet (February 2003) and dry (June 2003) seasons.

Kind of peanuts	Wet season						Dry season					
	Location of peanut sampling					Total of samples	Location of peanut sampling					
	Wonogiri regency			City of Surakarta			Wonogiri regency			City of Surakarta		
	Eromoko district	Ngadirojo district	Wonogiri district	Gede market	Legi market		Eromoko district	Ngadirojo district	Wonogiri district	Gede market	Legi market	
Wet raw pod	8	8	8	-	-	24	8	8	8	-	-	
Dry raw pod	6	9	9	-	-	24	6	12	6	-	-	
Raw kernel	6	9	12	15	12	54	6	9	12	15	12	
Total	20	26	29	15	12	102	20	29	26	15	12	

Moisture contents, physical quality of kernels, the incidence of *A. flavus* and aflatoxin B1 contamination

Moisture contents

Range and mean of moisture contents of kernels derived from various kinds of peanuts collected during the wet and dry seasons are presented in Tables 2 and 3, and Figure 1.

During both the wet and dry seasons, moisture contents of kernels in wet raw pods collected from farmers were higher than those of dry raw pods collected from collectors and raw kernels collected from retailers (Figure 1). Their moisture contents were very high (more than 30%), because in general the peanuts were harvested early, so they were still relatively immature. The major reasons for early harvesting are associated with the relative sweetness of kernels which are favoured by consumers, as well as the desire by farmers to obtain cash to allow the planting of the next crop. There is only minor interest in leaving the crop in the ground until full maturity is reached. Peanut kernels with high moisture contents will often produce shriveled kernels after drying.

The moisture contents of wet and dry pods and raw kernels collected during the wet season were higher than those collected during the dry season (Figure 1). The range and mean moisture contents of kernels in wet raw pods collected from farmers during the wet season were 39.6 – 50.3% and 43.2%, respectively; while those of kernels in dry raw pods from collectors were 9.2 – 35.6% and 13.3%, respectively; and those of raw kernels collected from retailers were 8.3 – 14.4% and 10.9%, respectively. The corresponding results for the dry season were 35.9 – 46.6% and 42.7%, 7.1 – 15.8% and 9.4%, 5.1 – 13.8% and 8.7%, respectively (Tables 2 and 3). Standar Nasional Indonesia (1995) determined that the safe moisture contents for storage of peanut pods and kernels were 9 and 8%, respectively.

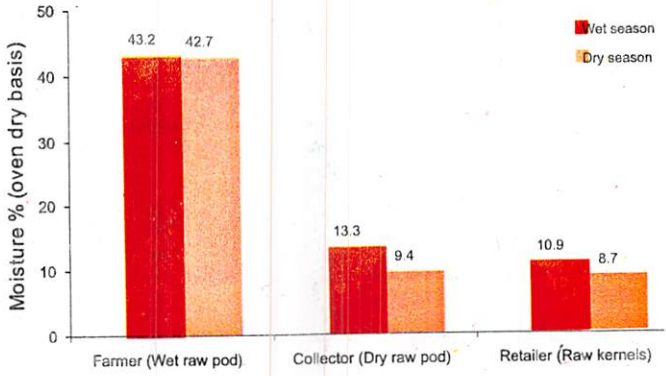

Figure 1. Mean moisture contents of peanut kernels derived from various kinds of peanuts collected from different points of the delivery chains during the wet and dry seasons.

Table 2. Moisture contents, physical quality of kernels, *Aspergillus flavus* infection, and aflatoxin B1 content of peanut kernels derived from various kinds of peanuts collected from different points of the delivery chains during the wet season (February 2003).

Peanut delivery chain	Kind of peanuts	Wet season						Dry season			
		Location of peanut sampling						Location of peanut sampling			
		Wonogiri regency			City of Surakarta		Total of samples	Wonogiri regency			City of S
		Eromoko district	Ngadirojo district	Wonogiri district	Gede market	Legi market		Eromoko district	Ngadirojo district	Wonogiri district	Gede market
Farmer	Wet raw pod	8	8	8	-	-	24	8	8	8	-
Collector	Dry raw pod	6	9	9	-	-	24	6	12	6	-
Retailer	Raw kernel	6	9	12	15	12	54	6	9	12	15
Total		20	26	29	15	12	102	20	29	26	15

Table 3. Moisture contents, physical quality of kernels, *Aspergillus flavus* infection, and aflatoxin B1 content of peanut kernels derived from various kinds of peanuts collected from different points of the delivery chains during the dry season (June 2003).

Peanut delivery chain	Kind of Peanuts	Number of samples	Range (mean) of moisture content (Based on % wet basis)	Physical quality of kernels			Number (%) of infected samples	Range of % infection in infected samples	Mean % infect kernel infect samp
				Range (mean) of % intact kernels	Range (mean) of % shriveled kernels	Range (mean) of % damaged kernels			
Farmer	Wet raw pod	24	39.6 – 50.3 (43.2)	25.5 – 84.5 (69.0)	7.8 – 70.5 (26.1)	2.7 – 8.0 (4.9)	20 (83.3)	1 – 64	6.6
Collector	Dry raw pod	24	9.2 – 35.6 (13.3)	25.8 – 82.0 (58.7)	11.7 – 68.5 (35.9)	3.0 –11.2 (5.4)	21 (87.5)	1 – 91	18.8
Retailer	Raw kernels	54	8.3 – 14.4 (10.9)	29.0 – 79.2 (63.5)	14.8 – 62.3 (29.4)	2.7 – 13.9 (7.1)	53 (98.2)	2 – 96	46.2

Physical quality of kernels

In general the intact kernels in peanut samples collected from various points in delivery chains during the wet and dry seasons were the highest, followed by shriveled and damaged kernels (Tables 2 and 3, Figure 2).

During the wet and dry seasons, mean percentages of damaged kernels of peanut samples collected from farmers were the lowest, while those collected from retailers were the highest (Figure 2). In general proportions of damaged kernels in peanut samples collected during the wet season were higher than those collected during the dry season. It was probably due to the higher pod moisture contents in the wet season which could have resulted in higher levels of fungal infection.

Mean percentages of damaged kernels of peanut samples collected from farmers, collectors and retailers during the wet and dry seasons were 4.9 and 3.9%, 5.4 and 4.0%, and 7.1 and 6.5%, respectively.

The incidence of A. flavus

The percentage of samples infected by *A. flavus*, range and mean percentages of infected kernels in infected samples of peanuts collected during the wet and dry seasons are presented in Tables 2 and 3, respectively, and Figures 3 and 4. During the wet and dry seasons, *A. flavus* infection and number infected kernels were highest in infected samples of peanuts collected from retailers, followed by those collected from collectors and farmers (Figure 3). The high levels of *A. flavus* infection in peanut samples collected from retailers was related to the post-harvest handling methods by players of the supply chain as well as the storage conditions. The existence of infected kernels in infected peanuts samples collected from farmers (6.6 and 2.5% during the wet and dry seasons, respectively) (Figure 4) was due to the existence of *A. flavus* in the soils of peanut farms (Dharmaputra *et al.* 2003a).

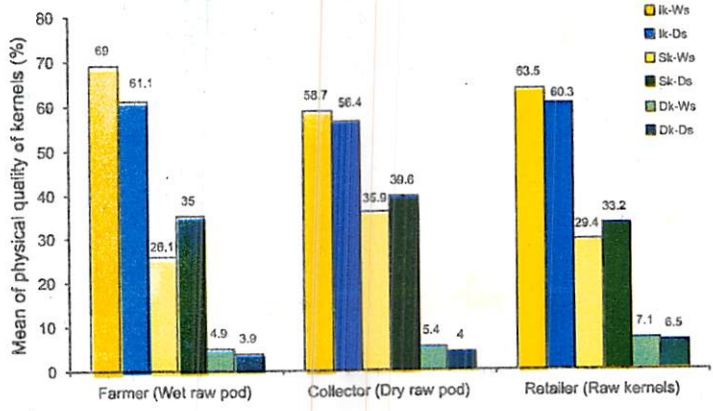

Figure 2. Mean percentages of physical quality of kernels. Peanut samples derived from various kinds of peanuts collected from different points of the delivery chains during the wet and dry seasons. Ik = intact kernels; Sk = shriveled kernels; Dk = damaged kernels; Ws = wet season, Ds = dry season

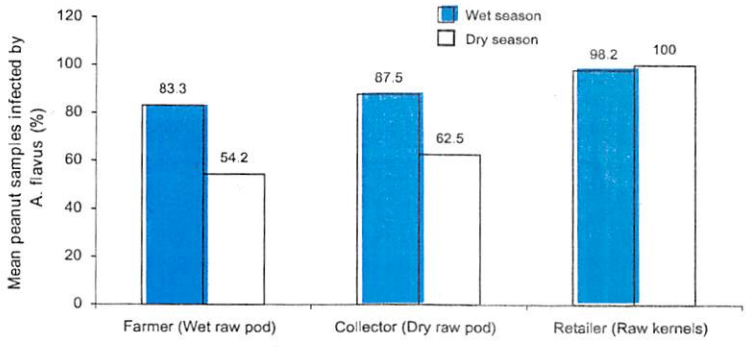

Figure 3. Mean percentage peanut samples infected by *Aspergillus flavus*. Peanut samples derived from various kinds of peanuts collected from different points of the delivery chains during the wet and dry seasons.

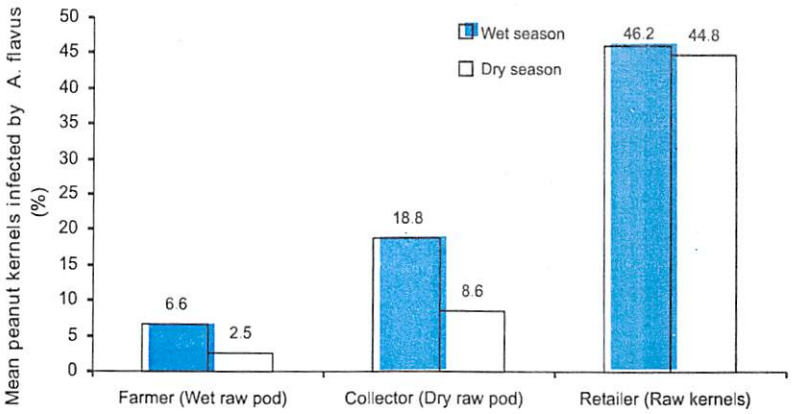

Figure 4. Mean percentage of kernels infected by *Aspergillus flavus* in infected samples. Peanut samples derived from various kinds of peanuts collected from different points of the delivery chains during the wet and dry seasons.

The levels of *A. flavus* infection and kernel infection in dry season were lower than the wet season (Figures 3 and 4). All most one hundred percent of raw kernel samples collected from retailers were infected by *A. flavus* during the wet and dry seasons (Figure 3). This high percentage was related to the methods of post - harvest

handling from farmers up to retailers. Moreover, peanut kernels are more easily infected by fungi compared to unshelled peanuts.

According to Dharmaputra and Retnowati (1996) the peanut kernels infected by *A. flavus* in samples collected from retailers in some locations in West Java during the wet season was 83 – 100%. Pitt *et al.* (1998) also reported high levels of *A. flavus* infection in i.e. 98% of 256 peanut kernel samples and 61% of all kernels examined derived from retailers in some locations in West and Central Java. According to Dharmaputra *et al.* (2003b) raw kernel samples collected from retailers in traditional markets located in Bogor, Pati, Yogyakarta and Malang had 100% infection with *A. flavus* during both the wet and dry seasons, respectively.

Aflatoxin B_1 contamination

The range of aflatoxin B_1 contents in kernels derived from various kinds of peanuts collected during the wet and dry seasons are presented in Tables 2 and 3. The levels of aflatoxin B_1 in wet raw pods during the wet and dry seasons were relatively low (Figures 5 and 6). This was most likely associated with 1) the low percentage of occurrence of toxigenic *A. flavus* in the soils of peanut fields, and hence the lower percentage of kernels infected by the toxigenic strains of *A. flavus*, 2) the high moisture content of kernels at harvest, 3) storage as pods rather than kernel as the presence of peanut shells protect the kernels from fungal infection. Dharmaputra *et al.* (2003b) also reported that the levels of aflatoxin B_1 in wet raw pods in the Pati region of Central Java were low. According to Heathcote (1984), minimum and maximum levels of aflatoxin in peanuts were obtained with kernel moisture contents of 9 – 10% and 25% at 30°C, respectively.

The range of aflatoxin B_1 contents in peanut samples collected from farmers, collectors and retailers during the wet season was higher than those collected during the dry season. Presumably higher humidity and more toxigenic *A. flavus* strains occurred in peanut samples collected during the wet season. During both the wet and dry seasons, the range of aflatoxin B_1 contents in peanut samples collected from retailers (< 3.6 – 1 859.3 and < 3.6 – 1 804.6 ppb during the wet and dry seasons, respectively) was the highest, followed by those collected from collectors (< 3.6 – 1 366.9 and 4.7 – 132.4 ppb during the wet and dry seasons, respectively) and farmers (< 3.6 – 93.9 and < 3.6 – 5.7 ppb during the wet and dry seasons, respectively) (Figures 5 and 6). According to Dharmaputra *et al.* (2003b) the highest aflatoxin B_1 contents were also found in peanuts collected from retailers in traditional markets located in Bogor, Pati, Yogyakarta and Malang.

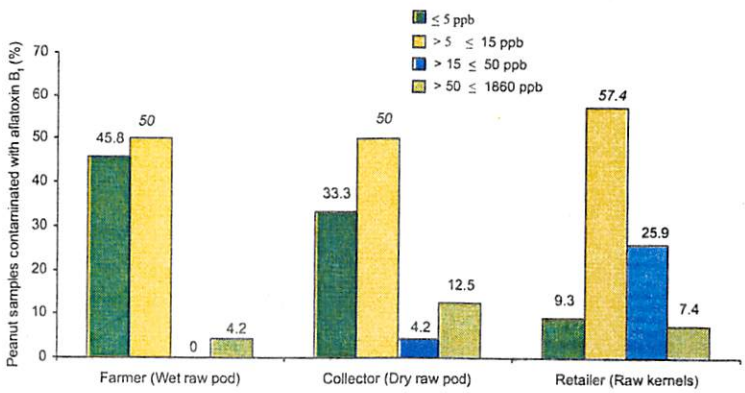

Figure 5. Percentage of peanut samples contaminated with different levels of aflatoxin B_1. Peanut samples were derived from various kinds of peanuts collected from different points of the delivery chains during the wet season.

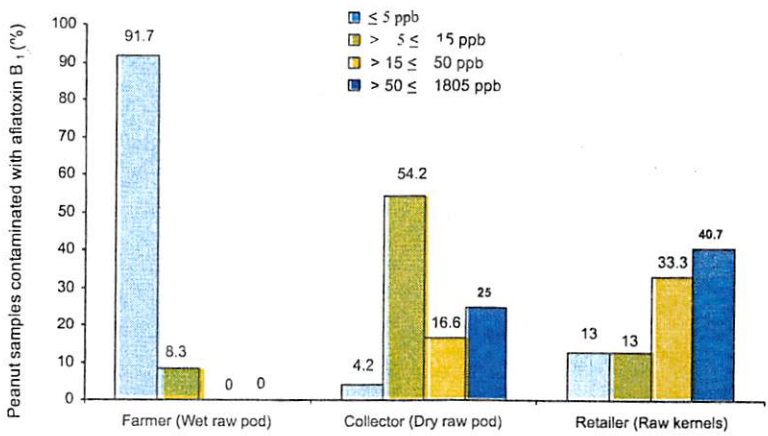

Figure 6. Percentage of peanut samples contaminated with different levels of aflatoxin B_1. Peanut samples were derived from various kinds of peanuts collected from different points of the delivery chains during the dry season.

In Australia the maximum allowable limit of aflatoxin in peanut and peanut products is 15 ppb (QDPI 2000). During the wet season, around 4, 17 and 33% of peanut samples collected from farmers, collectors and retailers, were respectively contaminated with more than 15 ppb of aflatoxin B_1 (Figure 5). During the dry season, around 42 and 74% of peanut samples collected from collectors and retailers were respectively contaminated with more than 15 ppb of aflatoxin B_1, while no wet raw pod samples collected from farmers were contaminated with more than 15 ppb (Figures 6). Our surveys of peanuts in the Indonesian food chain has clearly shown a) high levels of *A. flavus* contamination in pods and kernels which can produce aflatoxin rapidly under poor storage conditions (high humidity and temperature) which are very common in traditional peanut storages in Wonogiri region, and b) alarming levels of aflatoxin in a number of peanut products being sold in the retail market, and must accordingly result from poor post-harvest management and storage.

CONCLUSIONS

In Wonogiri regency most farmers sold peanuts to collectors after harvest in the form of wet raw pod, and most collectors sold peanuts to retailers in the form of dry raw kernels. The level of moisture content, physical quality of kernels, the incidence of *A. flavus* and aflatoxin B_1 contamination of peanuts depended on the status of product in the peanut delivery chain. Nevertheless, the highest percentages of samples infected by *A. flavus* and mean percentages of infected kernels in infected samples, and the highest aflatoxin B_1 contamination were found in raw kernels collected from retailers in traditional markets.

During the wet season, around 4, 17 and 33% of peanut samples collected from farmers, collectors and retailers, were respectively contaminated with more than 15 ppb of aflatoxin B_1. During the dry season, around 42 and 74% of peanut samples collected from collectors and retailers were respectively contaminated with more than 15 ppb of aflatoxin B_1, while no wet raw pod samples collected from farmers were contaminated with more than 15 ppb.

Post-harvest handling methods prior to peanuts being delivered to retailers especially at the retailer level in traditional markets will severely impact on the level of aflatoxin contamination in the Indonesian food chains.

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