## REVIEW ON AFLATOXIN IN INDONESIAN FOOD- AND FEEDSTUFFS AND THEIR PRODUCTS

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#### ABSTRACT

Aflatoxin is a human carcinogen that could contaminate food- and feedstuffs, and hence is a major food quality problem throughout the world. Afiatoxin is produced by certain strains of *AspergillusJlavus* and //. *parasiticus*. A number of studies have been carried out in Indonesia on atlatoxin contamination in Indonesian food- and feedstuffs and their products from 1990 up to present. They were maize, maize product, peanuts, soybean and soybean meal, black and white pepper, feed ingredients; chicken and duck feeds. Samples were collected from farmers, traders (middlemen), retailers (markets), supermarkets, exporters; poultry and duck community-based farms; and feed mill industries. High levels of aflatoxins were often found in maize, peanuts, chicken feed derived from markets, and duck feed. Low levels of aflatoxins were found in soybean meal and chicken feedstuff. Aflatoxins were not detected in soybean, black and white pepper.

Other studies have also been carried out on the effect of carbondioxide (CO2), phosphine, black pepper extract and antagonistic fungi on aflatoxin production of *A. flavus in vitro\* and the effect of airtight storage, phosphine, ammonium hydroxide, fermentation process, bag types, and phosphine in combination with different bag types on atlatoxin contents of maize, peanuts and soybean meal. Some of these methods reduced aflatoxin contents significantly.

Keywords: Aspergillus flavus I Aflatoxin / Food-and feed stuffs / Product

#### INTRODUCTION

Aflatoxin is a human carcinogen that contaminates food-and feedstuffs, produced by the common fungi *Aspergillus flavus* and *A. parasiticus*. The aflatoxin problem is a worldwide phenomenon, but it is particularly severe in developing countries, where food safety and security systems are not well developed to protect consumers against unsafe food products. In more developed countries, consumers are more aware of food safety issues such as aflatoxin, and are increasingly demanding those foods meet strict regulatory standards.

Aflatoxin limits for some commodities by importing countries are presented in Table 1. Among the foodstuff, peanuts is easily contaminated with aflatoxin. Based on the report of the 23<sup>rd</sup> Session of the Joint FAp/WHO Food Standards Programme, held in Rome, Italy, 28 June - 3 July 1999, Codex Alimentarius Commission adopted the maximum level of total aflatoxins in peanuts intended for further processing at 15 ppb.

This paper reviews the researches that have been undertaken in Indonesia from 1990 to date on aflatoxin contamination in food- and feedstuffs and their products, as well as control *ofA.flavus* and aflatoxin.

Table 1. Aflatoxin limits (ppb) for some commodities by importing countries (Lubulwa and Davis 1994).

Country	Aflatoxins	Peanuts	Nut, cereals	Maize and maize products	Feeds for dairy and young cattle and pigs	Feedstuffs for pigs and poultry
USA	B <sub>1</sub> +B <sub>2</sub> +G <sub>1</sub> +G <sub>2</sub>	20	20	20	20	20
Japan	$B_1$	10	10	10	10	20
European Community	B <sub>1</sub> +B <sub>2</sub> +G <sub>1</sub> +G <sub>2</sub>	5 to 50	1 to 30	5 to 50	10	20
France	B <sub>1</sub> +B <sub>2</sub> +G <sub>1</sub> +G <sub>2</sub>	0.1 (nut pastes)	5	10	5	10
Germany	$B_1$	5	2		5	10
Netherlands	$\mathrm{B}_1$	50			5	10
Britain	B <sub>1</sub> +B <sub>2</sub> +G <sub>1</sub> +G <sub>2</sub>	10	10	10		

#### AFLATOXIN IN FOOD-AND FEEDSTUFFS AND THEIR PRODUCTS

### Aflatoxin in maize and maize products

In Indonesia, maize is the second most important crop after rice. Purwoko *et al.* (1991) studied aflatoxin content of 34 maize samples collected from poultry farms and poultry feedmills located around Jakarta and Bogor. Aflatoxins were found in 91% of maize samples, and the predominant form was aflatoxin B|. The total concentration of aflatoxins in maize samples ranged from 22 to 6171 ppb, of which 91% contained aflatoxin B $_h$  ranging from 22 to 4074 ppb, while aflatoxin B $_2$  (71%) ranged from 11 to 3021 ppb. Aflatoxin G, was present in two samples, at concentration of 101 and 528 ppb, and only one sample contained aflatoxin G $_2$  at concentration of 144 ppb.

Maryam (1994) studied aflatoxin contamination of 32 maize samples derived from diagnostic samples sent to the Research Institute for Veterinary Science,

Bogor. It was reported that the percentage of maize samples contaminated with aflatoxin B,,  $B_2$ , G, and  $G_2$  were 78.1, 78.1, 20.0 and 20.0%, respectively. Their ranges were between 1.1 - 63.7, 0.5 - 12.6, 1.2 - 51.7, and 0.6 - 4.2 ppb, respectively (Table 2).

Table 2. Percentage, range and average of aflatoxin contents of maize derived from diagnostic samples sent to RIVS (Maryam 1994).

Aflatoxins	Contaminated samples (%)	Range (ppb)	Average (ppb)
Aflatoxin B <sub>1</sub>	78.12	1.1 - 63.7	11.7
Aflatoxin B <sub>2</sub>	78.12	0.5 - 12.6	2.5
Aflatoxin G <sub>1</sub>	20.00	1.2 - 51.7	1.9
Aflatoxin G <sub>2</sub>	20.00	0.6 - 4.2	0.2

A study by Dharmaputra *et al.* (1995) on aflatoxin content of 35 maize samples collected from farmers and traders in Lampung province in November 1992 reported that all of the samples (100%) contained aflatoxin B<sub>I</sub>, and 31% contained aflatoxin B<sub>2</sub> (Table 3). Eleven samples obtained from farmers contained 25.4 - 367 ppb of aflatoxin B<sub>h</sub> and 2 samples contained 12.5 and 12.7 ppb of aflatoxin B<sub>2</sub>, respectively; 4 samples obtained from village traders contained 119.7 - 276.7 ppb of aflatoxin B! and 11.9 - 41.6 ppb of aflatoxin B<sub>2</sub>, 4 samples obtained from middle traders contained only aflatoxin B! (25.6 - 97.6 ppb); 16 samples of big traders contained 23.4 - 278.7 ppb of aflatoxin B, and 5 samples contained 24.2 - 61.2 ppb of aflatoxin B<sub>2</sub>.

Table 1. Aflatoxin limits (ppb) for some commodities by importing countries (Lubulwa and Davis 1994).

Country	Aflatoxins	Peanuts	Nut, cereals	Maize and maize products	Feeds for dairy and young cattle and pigs	Feedstuffs for pigs and poultry
USA	B <sub>1</sub> +B <sub>2</sub> +G <sub>1</sub> +G <sub>2</sub>	20	20	20	20	20
Japan	$\mathbf{B}_1$	10	10	10	10	20
European Community	B <sub>1</sub> +B <sub>2</sub> +G <sub>1</sub> +G <sub>2</sub>	5 to 50	1 to 30	5 to 50	10	20
France	B <sub>1</sub> +B <sub>2</sub> +G <sub>1</sub> +G <sub>2</sub>	0.1 (nut pastes)	5	10	5	10
Germany	$\mathrm{B}_1$	5	2		5	10
Netherlands	$B_1$	50			5	10
Britain	B <sub>1</sub> +B <sub>2</sub> +G <sub>1</sub> +G <sub>2</sub>	10	10	10		

Sample		Maize	No. of	Aflatoxin content (ppb)			
number	Source	type	sample	$B_1$	B <sub>2</sub>	Total B	
11		Cob		79.4	0	79.4	
12	Village trader	Shelled	4	163.7	23.5	168.7	
13		Shelled		194.1	24.1	199.2	
14		Shelled		276.7	41.6	285.6	
15		Shelled		119.7	11.9	112.2	
16	Middle trader	Shelled	4	97.6	0	97.6	
17		Shelled		25.6	0	25.6	
18		Cob		27.4	0	27.4	
19		Cob		92.2	0	92.2	
20	Big trader	Shelled	16	46.8	0	46.8	
21		Shelled		46.8	. 0	46.8	
22		Shelled		100.8	40.7	109.5	
23		Shelled		45.7	0	45.7	
24		Shelled		91.5	0	91.5	
25		Shelled		46.4	0	46.4	
26		Shelled		138.9	44.8	148.4	
27		Shelled		117.1	0	117.1	
28		Shelled		48.1	0	48.1	
29		Shelled		167.4	24.2	172.55	
30		Shelled		23.4	0	23.4	
31		Shelled		278.7	61.2	291.72	
32		Shelled		91.2	0	91.2	
33		Shelled		140.9	0	140.9	
34		Shelled		69.1	0	69.1	
35		Shelled		117.2	25.4	122.60	

Another study on aflatoxin content of 108 maize samples collected from farmers, and 32 samples from village traders during the dry and wet seasons in Central Lampung (Lampung province) and Kediri (East Java province) regencies was conducted in 1993 - 1994 (Dharmaputra *et al.* 1996a). At farmers level, the total aflatoxin B| contents was between 5.3 - 291.4 ppb, while at village trader level, between 9.7 - 115.2 ppb (Table 4).

Table 4. Range of total aflatoxin B<sub>1</sub> content on maize at farmer and trader levels during the dry and wet seasons in Central Lampung and Kediri regencies (Dharmaputra et al. 1996a).

Location	Season	Total aflator	kin B <sub>1</sub> (ppb)
Location	Season	Farmer	Trader
Central Lampung	Dry	5.3 - 291.4	11.3 - 104.2
	Wet	8.6 - 153.5	20.6 - 69.0
Kediri	Dry	31 - 119.7	9.7 - 92.5
	Wet	11.4 - 282.7	20.8 - 115.2

A study by Dharmaputra and Putri (1996b) on total aflatoxin B, content in 11 samples of maize and maize products collected from some supermarkets in Bogor, West Java, reported that 1 sample of popcorn contained 15 ppb of the toxin; 1 sample of maizena flour contained 20 ppb of the toxin; 2 samples of maize for popcorn contained 40 and 40 ppb of the toxin, respectively; 1 sample of "maize chip" contained 19 ppb of the toxin. Non-detected aflatoxin was observed in 2 samples of popcorn, 1 sample of "marning", 2 samples of maize oil, and 1 sample of tortilla chips (Table 5).

Table 5. Aflatoxin content of maize and maize products collected from some supermarkets in Bogor (Dharmaputra and Putri 1996b).

Date of sampling	Maize and maize products	Total aflatoxin B <sub>1</sub> content (ppb)		
4 November 1994	Popcorn	15	- 66	
	Maizena	20		
	Maize for popcorn	40		
	Marning	0		
	Maize oil	- 0		
	Maize chip	19		
	Tortilla chips	0		
	Maize for popcorn	40		
	Popcorn	10		
12 November 1994	Popcorn	0		
	Maize oil	0		

### Aflatoxin in peanuts and peanut products

Peanuts is next to maize and soybeans as secondary crop of Indonesia. Dharmaputra *et al.* (1991) reported the aflatoxin contents in 35 peanut samples collected from 15 retailers located in three markets in Bogor, West Java, in September 1988. The aflatoxin contents were between 0 - 1154 ppb, while 80% of the peanut samples contained more than 30 ppb of aflatoxin.

Another study carried out by Haryadi and Setiastuty (1994) on aflatoxin contamination in 30 samples of raw and processed peanuts collected from various traders (big, medium and small) and processors during rainy and dry seasons in and around Bogor (West Java) and Denpasar (Bali), reported that aflatoxin B, was detected in 8 out of 15 samples collected during the rainy season, and in 5 samples collected during the dry season. Aflatoxin B<sub>2</sub> was detected in some samples contaminated with aflatoxin BI (Tables 6 and 7). The possibility of aflatoxin contamination was higher among small traders, although big traders cannot guarantee that their products were free from aflatoxin contamination. The study also revealed that in processed peanuts, aflatoxins could still be present.

Table 6. Aflatoxins contents in <u>raw</u> peanuts collected from various traders and processors during wet and dry seasons around Bogor and Denpasar (Haryadi and Setiastuty 1994).

			Afl	atoxins c	ontents (	ppb)			
Type and scale of business		Wet season			Dry season			5d 11	
		B <sub>1</sub>	B <sub>2</sub>	$G_1$	$G_2$	В	B <sub>2</sub>	G <sub>1</sub>	G <sub>2</sub>
Big traders	1	2.5		-		10.0	W.m.e	ang it	ш.
	2	10.0	-	-	-	-	-	-	-
Medium traders	1	-	-	-	-	-	-	-	-
	2	-		-	-	-	-		-
Small traders	1	5.0				-		-	-
	2	2.5	20.0	-	~	2.5	-	-	-
	3	5.0	-		-	5.0	-	-	-
	4	30.0	2	-	2	5.0	25.0	-	-
Processors	1	. 1	-	-		-	-	-	-
	2	10.0	2.5				-	-	_

Table 7. Aflatoxins contents in processed peanuts (Haryadi and Set
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				Afla	atoxins c	ontents (	opb)		
Type and scale of business		Wet season				Dry season			
		B <sub>1</sub>	$B_2$	$G_1$	$G_2$	$B_1$	$B_2$	G <sub>1</sub>	G <sub>2</sub>
Big traders	1	_	-		-		-	-	-
	2	15.0	5.0	-	-	2.5	-	-	- 5
Small traders	1	-	-	-		-	12	-	-
Processors	1	_	-	-		-	-		_
	2	-	-	-	-	-	-	_	_

Dharmaputra *et al.* (2002) reported that only one out of 15 samples of roasted pod and flour, and 14 samples of coated kernels of peanut collected from supermarkets in Bogor, Malang, Pati and Yogyakarta contained total aflatoxin of 15.54 and 20.72 ppb, respectively.

Table 8 shows the levels of aflatoxin found in maize and peanut samples from retailers located around Bogor and Yogyakarta. Forty five percent of 215 peanut samples contained more than 50 ppb of aflatoxin, 33% more than 300 ppb, and 22% exceeded 1000 ppb (Pitt and Hocking, 1996). Aflatoxin levels exceeding 300 ppb must be considered unsatisfactory, while those exceeding 1000 ppb must be considered totally unsatisfactory, capable of inducing acute toxic effects in both human and animals. An amount of 1% of maize and 17% of peanut samples, contained more than 1000 ppb aflatoxin.

Table 8. The percentage of maize and peanut samples contaminated with different levels of total aflatoxin content (Pitt and Hocking 1996).

Total aflatoxin content (range) (ppb)	Maize (%)	Peanuts (%)
≥5	68	44
> 5 ≤ 10	2	1
> 10 ≤ 50	8	10
> 50 ≤ 300	18	12
> 300 ≤ 1000	3	11
> 1000 < 5000	1	17
> 5000	0	5
Total no. of samples	96	215
Total production (t, 1991)	6409	920

### Aflatoxin in soybean and soybean meal

In Indonesia, soybean ranks next to maize in importance as secondary food crops. The protein content of soybean is relatively high (42 - 50%), thus soybean meal is an important component of feedstuff. Purwoko *et at.* (1991) studied aflatoxin contents of 10 soybean samples collected from some poultry farms and poultry feed mills located around Jakarta and Bogor in July 1990. Aflatoxins were not detected in the samples.

Dharmaputra *et al.* (1997) reported that only 5 soybean meal samples were contaminated by aflatoxin from a study on aflatoxin content of 13 soybean meal samples collected from 10 feed mills factories, three BULOG warehouses and one soybean crushing plant factory. The contents of aflatoxin were 7.9, 11.3, 14.5, 28.5 and 34 ppb, respectively. According to Venkitasubramanian (1977), phytic acid in soybeans can bind zinc ions, while zinc ions are the important elements of aflatoxin synthesis.

### Aflatoxin in pepper

Among spices, black and white pepper are important export commodities in Indonesia. Aflatoxins were not detected in 40 samples of black and white pepper (20 samples/commodity) collected from some retailers and supermarkets in Bogor, and some exporters in Bangka island and Lampung province (Dharmaputra *et al.* 1999).

#### Aflatoxin in chicken feed, duck feed, mixed duck feed, and rice bran

Dharmaputra and Putri (1996b) reported that the total aflatoxin B[ content of 39 chicken feed samples collected from 3 markets in Bogor during dry and wet seasons was between 0 — 200.73 ppb. Twenty six out of 39 samples (67%) contained aflatoxins more than 30 ppb.

A study by Zahari and Tarmudji (1995) on the aflatoxin content of 19 duck feed, 8 mixed duck feed and 8 rice bran samples collected from a duck farm in South Kalimantan reported that in duck feed 100, 100, 52.6 and 15.8% of the samples were contaminated with aflatoxins  $B_1$ ,  $B_2$ ,  $G_3$ , and  $G_4$ , respectively. Their contents were between  $G_4$ 0 - 160.0 ppb,  $G_4$ 8 - 60.0 ppb, 0 - 60.0 ppb, and 0 - 8.0 ppb, respectively. In mixed duck feed, only one sample was contaminated with aflatoxin  $G_4$ 8, and its content was 8.0 ppb. In rice bran, 75% of the samples were contaminated with aflatoxin  $G_4$ 9. Their contents were between 0 - 20.0 ppb. Two out of 8 samples (25%) contained 7.2 and 72.0 of aflatoxin  $G_4$ 9 was detected in all samples.

#### CONTROL OF ASPERGILLUS FLA VUS AND AFLATOXIN

## The effects of carbondioxide on mycelial growth and aflatoxin production of A. flavus in pure culture

Dharmaputra *et al.* (1992) studied the effects of CO<sub>2</sub> concentrations of 20, 40, 60 and 80% on mycelial growth and aflatoxin production of three isolates of *A. flavus*. As a control, these fungal isolates were maintained in air. The CO<sub>2</sub> concentrations used significantly affected both mycelial growth and aflatoxin production of *A. flavus* isolates. CO<sub>2</sub> at 20% started to inhibit the two parameters (Figures 1 and 2). The growth *of A. flavus* and aflatoxin production decreased with the increase of CO<sub>2</sub> concentrations.

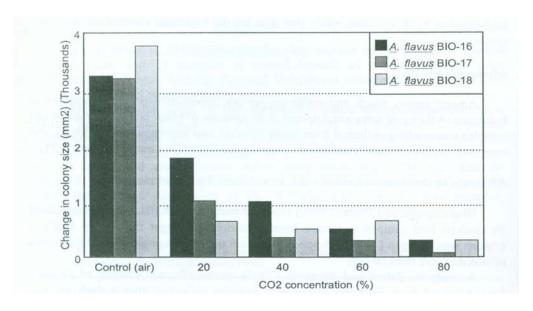
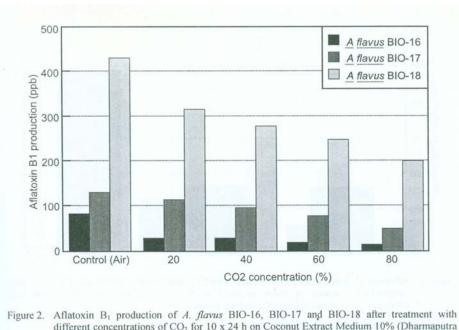


Figure 1. Mycelial growth of *A. flavus* BIO-16, BIO-17 and BIO-18 after treatment with different concentrations of CO<sub>2</sub> for 7 x 24 h on Potato Dextrose Agar (Dharmaputra *et al.* 1992).

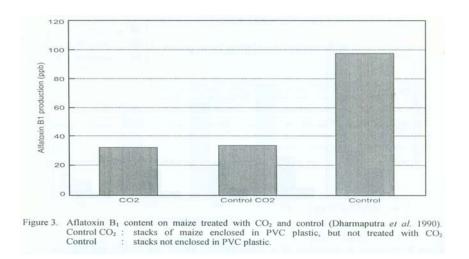


different concentrations of CO2 for 10 x 24 h on Coconut Extract Medium 10% (Dharmaputra et al. 1992).

#### The effect of carbondioxide on aflatoxin production in maize

An investigation was carried out by Dharmaputra et al. (1990) on the effect of CO<sub>2</sub> on aflatoxin production in stored maize. Stacks of stored maize were sealed with PVC sheeting and treated with CO<sub>2</sub> for storage periods varying from 10 to 120 days. The concentration of CO<sub>2</sub> used was 2.4 kg/t. The control groups consisted of both stacks of maize sealed in plastic sheets, but not treated with CO<sub>2</sub>, and stacks not sealed in plastic sheets.

The aflatoxin B, content of maize, whether in plastic sheets and treated with CO<sub>2</sub> or only in plastic sheets, was lower than that of the unsheeted and untreated stacks (Figure 3). The control showed that the aflatoxin content increased with the increasing duration of storage.



## The effect of phosphine on mycelial growth and aflatoxin production of A. flavus in pure culture

Dharmaputra *et al.* (1991) studied the effect of phosphine on mycelial growth and aflatoxin B, production of two *A. flavus* isolates. The concentrations of phosphine used were 0.5, 1.5, 2.5 and 3.5 mg/L. As a control, these fungal isolates were maintained in air.

The phosphine concentrations significantly affected both the mycelial growth and aflatoxin production of the isolates *of A. flavus*. Mycelial growth and aflatoxin production decreased with increasing phosphine concentrations (Figures 4 and 5). Inhibition of mycelial growth commenced at 0.5 mg/L (Figure 4). Although the two isolates were still able to produce aflatoxin after treatment with 3.5 mg/L of phosphine, the amounts were low (Figure 5).



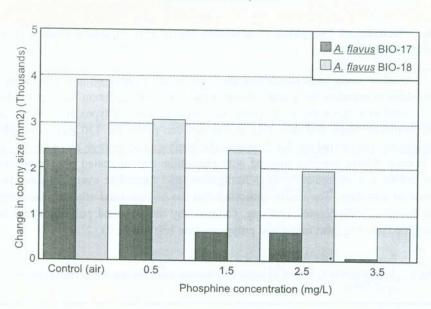


Figure 4. Mycelial growth of *A. flavus* BIO-16, BIO-17 and BIO-18 after treatment with different concentrations of phosphine for 5 x 24h on Potato Dextrose Agar (Dharmaputra *et al.* 1991).

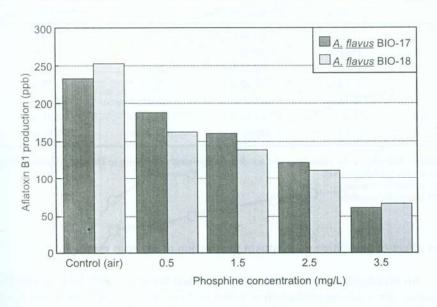


Figure 5. Aflatoxin B<sub>1</sub> production of A. flavus BIO-17 and BIO-18 after treatment with different concentrations of phosphine for 5 x 24 h on Coconut Extract medium 10% (Dharmaputra et al. 1991).

## The effectivity of phosphine to maintain the quality of maize packed in two different bag types

Another study was conducted by Putri et al. (1999) on the effectivity of phosphine to maintain the quality of maize packed in two different bag types. Maize with initial moisture content of  $\pm$  14% was stored in two bag types, namely jute and polypropylene bags, and then laid on the wooden pallet for 130 days. Phosphine fumigation was carried out for 5 days at the beginning of storage and after 95 days of storage. Maize samples (treated with phosphine and untreated one) were taken just before and immediately after furnigation, and thereafter every 30 days for a period of 130 days. The results indicated that the highest total aflatoxin B, content was found in unfumigated jute bag, followed by unfumigated polypropylene bag, fumigated jute bag, and fumigated polypropylene bag (Tab'le 9).

Table 9.	Total aflatoxin B <sub>1</sub> content on maize in unfumigated and fumigated bags during storage (Put	tri
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Storage – duration (days) –	Total aflatoxin B <sub>1</sub> content (ppb)							
	Ur	fumigated	Fumigated					
	Jute bag	Polypropylene bag	Jute bag	Polypropylene bag				
O a)	17.44 n	17.49 n	17.40 n	17.49 n				
5	23.31 n	20.48 n	19.48 n	17.57 n				
35	48.92 kl	42,75 lm	20.28 lm	35.22 m				
65	70.14 hi	61.62 ij	58.43 ijk	52.78 jkl				
95 b)	109.13 cd	105.00 cd	87.28 ef	74.62 gh				
100	121.80 Ь	117.16 bc	97.42 de	82.91 fg				
130	149.62 a	140.65 a	117.26 bc	107.59 cd				

Numbers followed by the same letter did not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

a) 1st phosphine treatment

b) 2nd phosphine treatment

#### The effect of phosphine on aflatoxin production of soybean meal

An investigation was carried out by Dharmaputra et al. (1993) on the effect of phosphine on aflatoxin production of stored soybean meal. Soybean meal was stored in polypropylene bags for 190 days. Four stacks were treated with phosphine (2.1 g/t) for 5 days, once at the beginning of storage and again after 95 days of storage. Four untreated stacks served as control.

There was a significant difference in aflatoxin B, content between the treated and the untreated samples (Table 10). Aflatoxin B, content increased during prolonged storage in both treated an untreated soybean meal (Figure 6). The results indicated that phosphine somehow inhibits aflatoxin BI production.

Aflatoxin B<sub>1</sub> content on soybean meal treated with phosphine and control (Dharmaputra et Table 10. al. 1993).

Storage duration (days)	Aflatoxin B <sub>1</sub> content (ppb)		E volue
	Control	Phosphine	- F-value
O a)	5.82	0.00	E lintor in the
5	14.49	9.42	0.46
35	18.35	9.92	14.77 *)
65	18.42	11.21	23.50 **
95 b)	22.42	12.67	10,255.91 **
100	24.94	14.06	32,828.57 **
130	27.85	14.84	497.96 **
160	30.25	15.28	1,625.72 **
190	31.12	23.55	10.33 *)

<sup>1&</sup>lt;sup>st</sup> phosphine treatment 2<sup>nd</sup> phosphine treatment b)

Significant difference at 99% confidence level

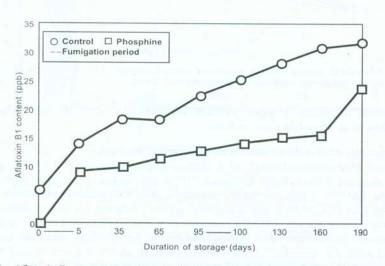


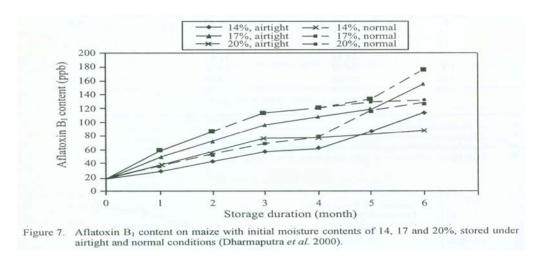
Figure 6. Aflatoxin B<sub>1</sub> content on soybean meal treated with phosphine and control (Dharmaputra et al.

Significant difference at 95% confidence level

### The effect of airtight storage on aflatoxin production in maize

An investigation was carried out by Dharmaputra *et al.* (2000) on the effect of airtight storage on aflatoxin production in maize. The maize was placed in polyethylene bag under airtight condition (initial  $O_2$  content was  $1.4 \pm 0.1\%$ ), and stored for six months under laboratory conditions. The initial moisture contents of maize were  $14 \pm 0.3$ ,  $17 \pm 0.2$  and  $20 \pm 0.2\%$ . As control, maize with the same initial moisture contents was placed in polyethylene bag under normal condition (air) with  $O_2$  content of 21%.

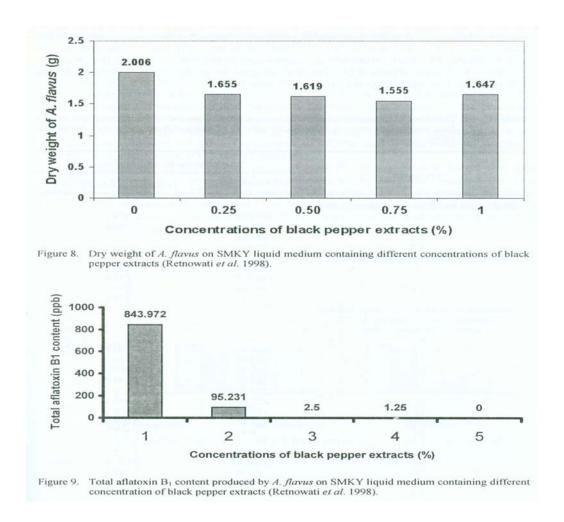
The total aflatoxin B, content on maize with the three initial moisture contents packed under normal condition was higher than under airtight condition (Figure 7). The total aflatoxin B! content increased with the increase of storage duration.



# The effects of black pepper extract on mycelial growth and aflatoxin production of A. flavus in pure culture

Retnowati *et al.* (1998) studied the effects of black pepper extract on mycelial growth and aflatoxin production of *A. flavus*. The concentrations of black pepper extracts used were 0.25, 0.50, 0.75 and 1.00% v/v media. *Aspergillus flavus* was grown on SMKY liquid medium containing different concentrations of black pepper. As a control, SMKY liquid medium did not contain black pepper extract.

Black pepper extract at 0.25% concentration started to inhibit mycelial growth and aflatoxin production of *A. flavus*. Mycelial growth and aflatoxin contents decreased with the increase of the extract concentrations (Figures 8 and 9). No aflatoxin was detected on SMKY liquid medium containing 1% black pepper extract.



## The effect of ammonium hydroxide on aflatoxin production of maize

The effect of ammonium hydroxide on aflatoxin production of maize stored under laboratory conditions was investigated by Dharmaputra and Ambarwati

(1999). Maize var. Arjuna was treated with different concentrations of ammonium hydroxide, i.e. 0.5, 1.0, and 1.5% (v/v). The durations of the treatments were 12, 24 and 36 hours. The result showed that ammonium hydroxide decreased the aflatoxin content. The lowest aflatoxin content was detected when the maize was treated with 1.5% ammonium hydroxide for 36 hours (Table 11).

Table 11. The effect of ammonium hydroxide concentrations, duration of ammonia treatment, and their interactions on total aflatoxin B<sub>1</sub> content of maize (Dharmaputra and Ambarwati 1999).

	Total aflatoxin B <sub>1</sub> content (ppb)		
Treatment	Not transformed	Transformed into log x + 1	
Ammonium hydroxide concentrations (%)			
0	218.070	2.338 a	
0.5	89.113	1.599 b	
1.0	84.548	1.537 c	
1.5	73.013	1.349 d	
Duration of ammonia treatment (hours)			
0	220.895	3.342 e	
12	124.778	2.051 f	
24	59.628	1.296 g	
36	59.445	1.136 h	
nteraction between ammonium hydroxide concentrations (%) and duration of ammonia treatment (hours)			
0	220.95	2.342 i	
12	220.89	2.344 i	
24	209.57	2.322 i	
36	220.88	2.344 i	
0.5			
0	220.90	2.342 i	
12	115.27	2.062 j	
24	11.87	1.074 m	
36 1.0	8.41	0.922 o	
0	220.88	2.342 i	
12	100.74	2.003 k	
24	10.49	1.021 n	
36	6.08	0.784 o	
1.5			
0	220.85	2.342 i	
12	62.21	1.794 i	
24	5.85	0.767 o	
36	3.14	0.496 p	

Numbers followed by the same letter do not differ significantly according to Duncan's Multiple Range Test at 95% confidence level.

### The effect of fermentation process on the aflatoxin production of peanuts

The effect of traditional fermentation process on aflatoxin production of peanuts was studied by Edi *et al.* (1990) and Fardiaz *et al.* (1993). Peanuts were contaminated with aflatoxin-producing *A. flaws*, and incubated at 30°C for 6 days. The oil was extracted by hydraulic pressure, and the peanut presscake processed to make a fermented product called "oncom". To make "bla'ck oncom", the presscake was fermented with *Rhizopus oligosporus*, while to make "red oncom" the presscake was fermentated with *Nenrospora sitophila*. Soaking of peanut presscake in water for 24 hours reduced the aflatoxin content to 48.6%, and it was reduced further to 42.9% after steaming at 95°C for 90 minutes.

Fermentation of the peanut presscake by *R. oligosporus* reduced the total aflatoxin content to 13.4%, while fermentation by *N. sitophila* reduced the total aflatoxin content to 41.1% of the original aflatoxin content in peanut presscake (Figure 10). Aflatoxin B, appeared to be the most sensitive to soaking and steaming processes. The decrease in aflatoxin content during soaking and steaming might be due to leaching, since aflatoxin is relatively resistant to heat.

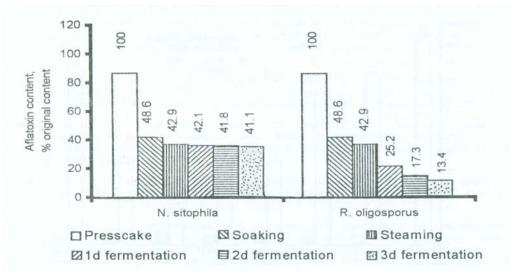


Figure 10. The effect of fermentation process on aflatoxin content of peanuts (Edi et al. 1990; Fardiaz et al. 1993).

#### The effect of bag types on aflatoxin production of peanuts

Bulaong and Dharmaputra (2002) studied the effect of four bag types namely jute bag, poly-propylene bag, jute bag lined with thin polyethylene (PE), and jute bag lined with thick PE, on aflatoxin production of peanuts with initial moisture content of 8%. Storage was done for 6 months under warehouse conditions.

No significant differences were obtained in aflatoxin levels among bag types, but at the end of six months storage, aflatoxin contents of peanuts packed in jute bag, polypropylene bag, jute bag lined with thin PE, and jute bag lined with thick PE were 31.5, 23.1, 16.8 and 18.9 ppb, respectively. Based on the results, the immediate packaging of dried shelled peanuts at safe moisture level in jute bag lined with thin PE is recommended. The water vapor transmission rate of the thin PE is  $1 \text{ g/m}^2/24 \text{ h}$ .

### The effect of antagonistic fungi on aflatoxin production of A. flavus in vitro

Investigation was carried out by Dharmaputra *et al.* (2001) on the effect of fungi isolated from soil of peanut fields at Wonogiri regency, Central Java, on aflatoxin production of *A. flavus*. Figure 11 shows that *A. niger* was the most promising fungal antagonist, because it caused the highest percent inhibition (80%) of aflatoxin production *of A. flavus* isolate 10<sub>2</sub>, followed by non-toxigenic *A. flavus* isolate 61<sub>2</sub> (61%), *A. tamarii* (60%) and non-toxigenic *A. flavus* isolate 36, (59%).

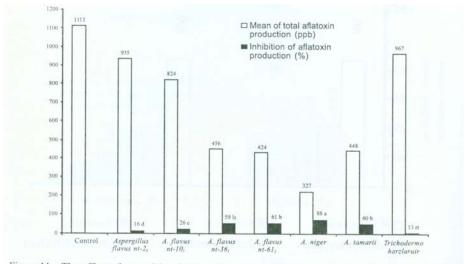


Figure 11. The effect of promising fungal antagonist on aflatoxin production of toxigenic A. flavus isolate 102 and the percentage of inhibition (Dharmaputra et al. 2001).

#### **CONCLUSIONS**

Only very limited analyses and surveys on the extent of aflatoxin contamination in Indonesian food- and feedstuffs and their products have been carried out. High levels of aflatoxins were often found in maize and peanuts. Consequently,

researches have been concentrated on the effects of postharvest handling and fumigation on fungal infection and aflatoxin production in those two commodities. In soybean meal, aflatoxin contents were relatively low.

No research has been carried out on aflatoxin contamination in relation with human health, cultural practices, peanut trading and industry. It is essential that a rigorous assessment of the critical points of entry of aflatoxin into the food and feed chains should be determined to more clearly assess the importance of pre-versus post-harvest management strategies.

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