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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. Aflatoxin is produced by certain strains of *Aspergillus flavus* and *A. parasiticus*. A number of studies have been carried out in Indonesia on aflatoxin 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 carbon dioxide (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 aflatoxin contents of maize, peanuts and soybean meal. Some of these methods reduced aflatoxin contents significantly.

Keywords: *Aspergillus flavus* / 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 23rd Session of the Joint FAO/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 of *A.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 ₁ +B ₂ +G ₁ +G ₂	20	20	20	20	20
Japan	B ₁	10	10	10	10	20
European Community	B ₁ +B ₂ +G ₁ +G ₂	5 to 50	1 to 30	5 to 50	10	20
France	B ₁ +B ₂ +G ₁ +G ₂	0.1 (nut pastes)	5	10	5	10
Germany	B ₁	5	2		5	10
Netherlands	B ₁	50			5	10
Britain	B ₁ +B ₂ +G ₁ +G ₂	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 B₁. The total concentration of aflatoxins in maize samples ranged from 22 to 6171 ppb, of which 91% contained aflatoxin B₁ ranging from 22 to 4074 ppb, while aflatoxin B₂ (71%) ranged from 11 to 3021 ppb. Aflatoxin G₁ was present in two samples, at concentration of 101 and 528 ppb, and only one sample contained aflatoxin G₂ 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 B₁, B₂, G₁, and G₂ 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 ₁	78.12	1.1 – 63.7	11.7
Aflatoxin B ₂	78.12	0.5 – 12.6	2.5
Aflatoxin G ₁	20.00	1.2 – 51.7	1.9
Aflatoxin G ₂	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 B₁, and 31% contained aflatoxin B₂ (Table 3). Eleven samples obtained from farmers contained 25.4 - 367 ppb of aflatoxin B₁ and 2 samples contained 12.5 and 12.7 ppb of aflatoxin B₂, respectively; 4 samples obtained from village traders contained 119.7 - 276.7 ppb of aflatoxin B₁ and 11.9 - 41.6 ppb of aflatoxin B₂, 4 samples obtained from middle traders contained only aflatoxin B₁ (25.6 - 97.6 ppb); 16 samples of big traders contained 23.4 - 278.7 ppb of aflatoxin B₁, and 5 samples contained 24.2 - 61.2 ppb of aflatoxin B₂.

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 ₁ +B ₂ +G ₁ +G ₂	20	20	20	20	20
Japan	B ₁	10	10	10	10	20
European Community	B ₁ +B ₂ +G ₁ +G ₂	5 to 50	1 to 30	5 to 50	10	20
France	B ₁ +B ₂ +G ₁ +G ₂	0.1 (nut pastes)	5	10	5	10
Germany	B ₁	5	2		5	10
Netherlands	B ₁	50			5	10
Britain	B ₁ +B ₂ +G ₁ +G ₂	10	10	10		

Table 3. Continued

Sample number	Source	Maize type	No. of sample	Aflatoxin content (ppb)		
				B ₁	B ₂	Total B ₁
11	Village trader	Cob	4	79.4	0	79.4
12		Shelled		163.7	23.5	168.7
13		Shelled		194.1	24.1	199.2
14		Shelled		276.7	41.6	285.6
15	Middle trader	Shelled	4	119.7	11.9	112.2
16		Shelled		97.6	0	97.6
17		Shelled		25.6	0	25.6
18		Cob		27.4	0	27.4
19	Big trader	Cob	16	92.2	0	92.2
20		Shelled		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		

Conversion factor B₁ = 4.7

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 B₁ 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 B₁ 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 aflatoxin B ₁ (ppb)	
		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 B₁ 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 ₁ content (ppb)
4 November 1994	Popcorn	15
	Maizena	20
	Maize for popcorn	40
	Marning	0
	Maize oil	0
	Maize chip	19
	Tortilla chips	0
	Maize for popcorn	40
12 November 1994	Popcorn	10
	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 B₁ was detected in 8 out of 15 samples collected during the rainy season, and in 5 samples collected during the dry season. Aflatoxin B₂ was detected in some samples contaminated with aflatoxin B₁ (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 raw peanuts collected from various traders and processors during wet and dry seasons around Bogor and Denpasar (Haryadi and Setiastuty 1994).

Type and scale of business		Aflatoxins contents (ppb)							
		Wet season				Dry season			
		B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂
Big traders	1	2.5	-	-	-	-	-	-	-
	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	-	-	-	5.0	25.0	-	-
Processors	1	-	-	-	-	-	-	-	-
	2	10.0	2.5	-	-	-	-	-	-

(-): undetected

Table 7. Aflatoxins contents in processed peanuts (Haryadi and Setiastuty 1994).

Type and scale of business		Aflatoxins contents (ppb)							
		Wet season				Dry season			
		B ₁	B ₂	G ₁	G ₂	B ₁	B ₂	G ₁	G ₂
Big traders	1	-	-	-	-	-	-	-	-
	2	15.0	5.0	-	-	2.5	-	-	-
Small traders	1	-	-	-	-	-	-	-	-
Processors	1	-	-	-	-	-	-	-	-
	2	-	-	-	-	-	-	-	-

(-) : undetected

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 al.* (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 B₁ 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 B₁, B₂, G₁, and G₂, respectively. Their contents were between 4.0 - 160.0 ppb, 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 B₁, and its content was 8.0 ppb. In rice bran, 75% of the samples were contaminated with aflatoxin B₁. Their contents were between 0 - 20.0 ppb. Two out of 8 samples (25%) contained 7.2 and 72.0 of aflatoxin B₂. Only one sample of rice bran was contaminated with aflatoxin G₁ (3.2 ppb). No aflatoxin G₂ was detected in all samples.

CONTROL OF *ASPERGILLUS FLAVUS* 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 CO₂ 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 CO₂ concentrations used significantly affected both mycelial growth and aflatoxin production of *A. flavus* isolates. CO₂ 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 CO₂ concentrations.

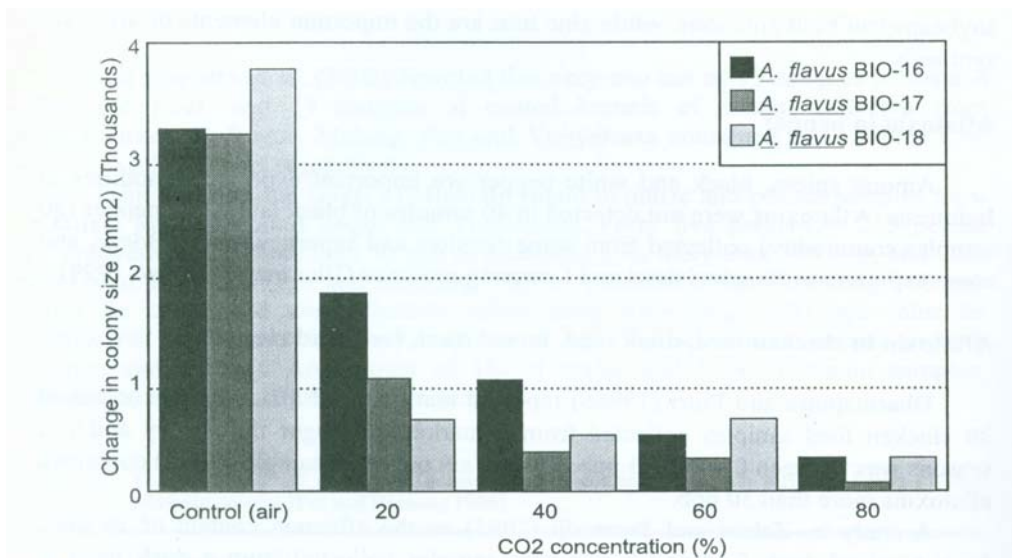
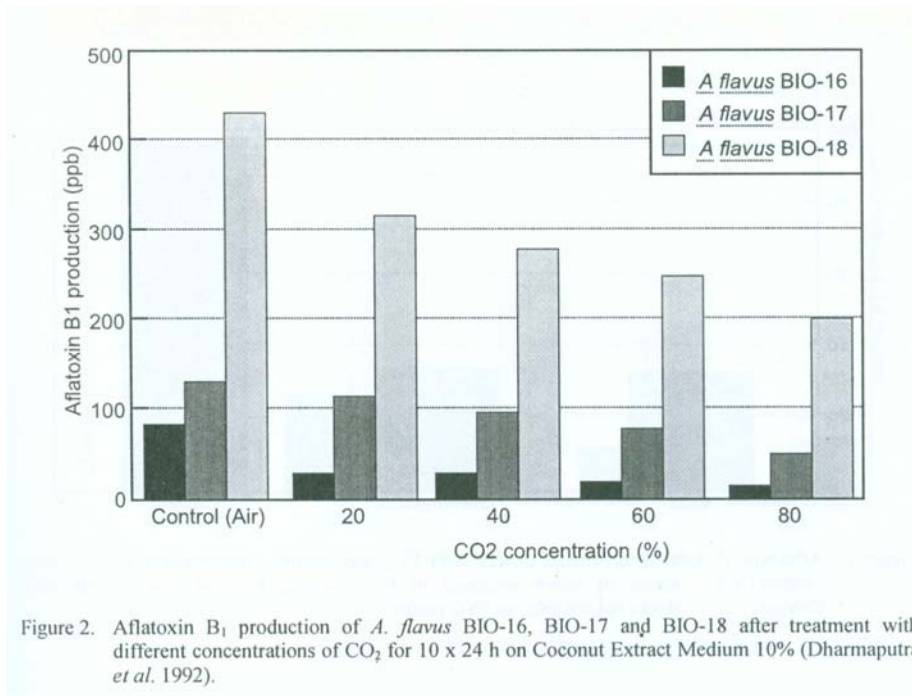


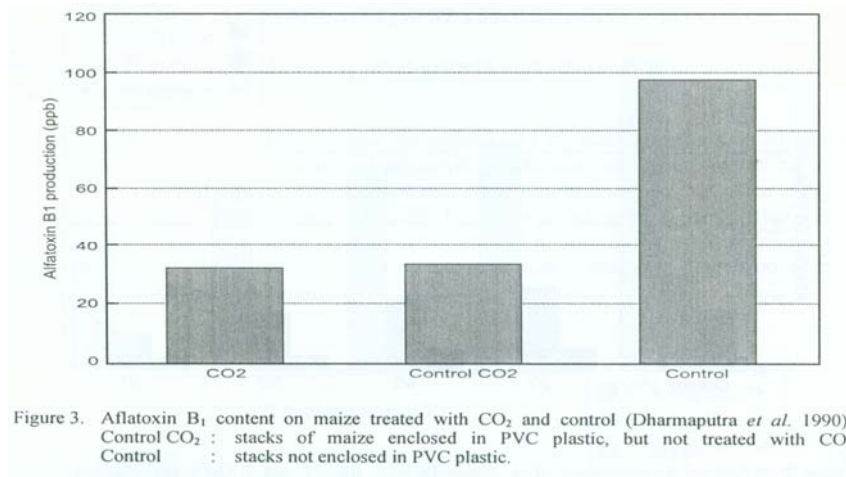
Figure 1. Mycelial growth of *A. flavus* BIO-16, BIO-17 and BIO-18 after treatment with different concentrations of CO₂ for 7 x 24 h on Potato Dextrose Agar (Dharmaputra *et al.* 1992).



The effect of carbondioxide on aflatoxin production in maize

An investigation was carried out by Dharmaputra *et al.* (1990) on the effect of CO₂ on aflatoxin production in stored maize. Stacks of stored maize were sealed with PVC sheeting and treated with CO₂ for storage periods varying from 10 to 120 days. The concentration of CO₂ used was 2.4 kg/t. The control groups consisted of both stacks of maize sealed in plastic sheets, but not treated with CO₂, and stacks not sealed in plastic sheets.

The aflatoxin B₁ content of maize, whether in plastic sheets and treated with CO₂ or only in plastic sheets, was lower than that of the unsealed 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 B₁ 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).

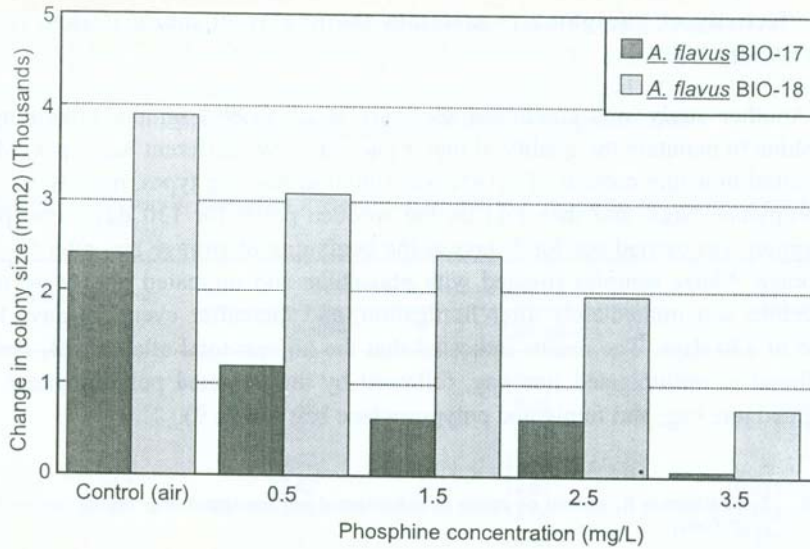


Figure 4. Mycelial growth of *A. flavus* BIO-16, BIO-17 and BIO-18 after treatment with different concentrations of phosphine for 5 x 24h on Potato Dextrose Agar (Dharmaputra *et al.* 1991).

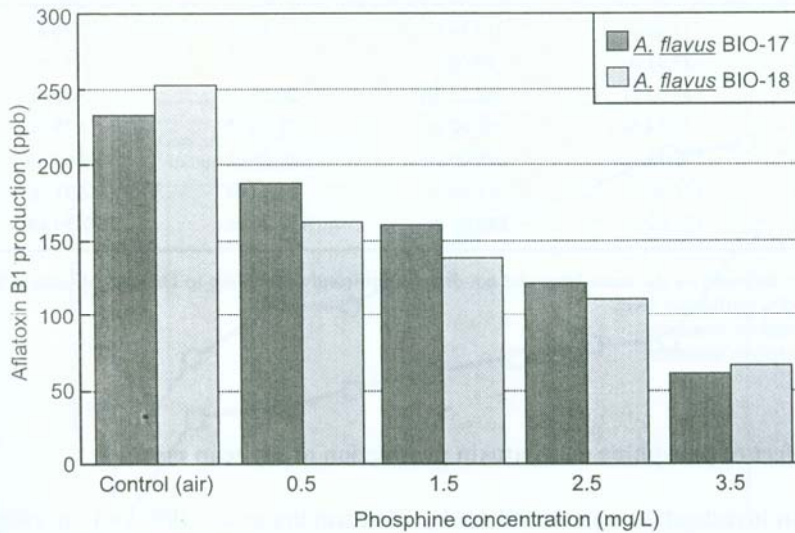


Figure 5. Aflatoxin B₁ production of *A. flavus* BIO-17 and BIO-18 after treatment with different concentrations of phosphine for 5 x 24 h on Coconut Extract medium 10% (Dharmaputra *et al.* 1991).

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 fumigation, and thereafter every 30 days for a period of 130 days. The results indicated that the highest total aflatoxin B₁ content was found in unfumigated jute bag, followed by unfumigated polypropylene bag, fumigated jute bag, and fumigated polypropylene bag (Table 9).

Table 9. Total aflatoxin B₁ content on maize in unfumigated and fumigated bags during storage (Putri *et al.* 1999).

Storage duration (days)	Total aflatoxin B ₁ content (ppb)			
	Unfumigated		Fumigated	
	Jute bag	Polypropylene bag	Jute bag	Polypropylene bag
0 ^{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 b	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 B₁ content between the treated and the untreated samples (Table 10). Aflatoxin B₁ content increased during prolonged storage in both treated and untreated soybean meal (Figure 6). The results indicated that phosphine somehow inhibits aflatoxin B₁ production.

Table 10. Aflatoxin B₁ content on soybean meal treated with phosphine and control (Dharmaputra *et al.* 1993).

Storage duration (days)	Aflatoxin B ₁ content (ppb)		F-value
	Control	Phosphine	
0 ^{a)}	5.82	0.00	
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 ^{*)}

a) 1st phosphine treatment

b) 2nd phosphine treatment

*) Significant difference at 95% confidence level

***) Significant difference at 99% confidence level

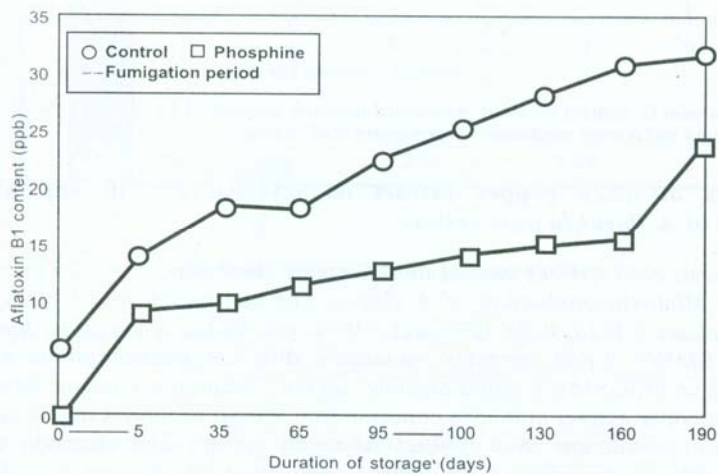


Figure 6. Aflatoxin B₁ content on soybean meal treated with phosphine and control (Dharmaputra *et al.* 1993).

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 O₂ content was 1.4 ± 0.1%), and stored for six months under laboratory conditions. The initial moisture contents of maize were 14 ± 0.3, 17 ± 0.2 and 20 ± 0.2%. As control, maize with the same initial moisture contents was placed in polyethylene bag under normal condition (air) with O₂ content of 21%.

The total aflatoxin B₁ content on maize with the three initial moisture contents packed under normal condition was higher than under airtight condition (Figure 7). The total aflatoxin B₁ content increased with the increase of storage duration.

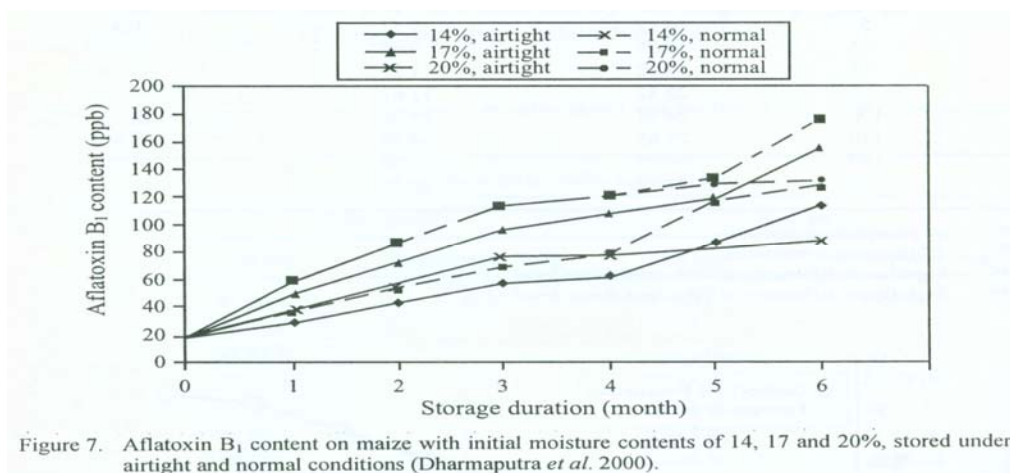


Figure 7. Aflatoxin B₁ content on maize with initial moisture contents of 14, 17 and 20%, stored under airtight and normal conditions (Dharmaputra *et al.* 2000).

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.

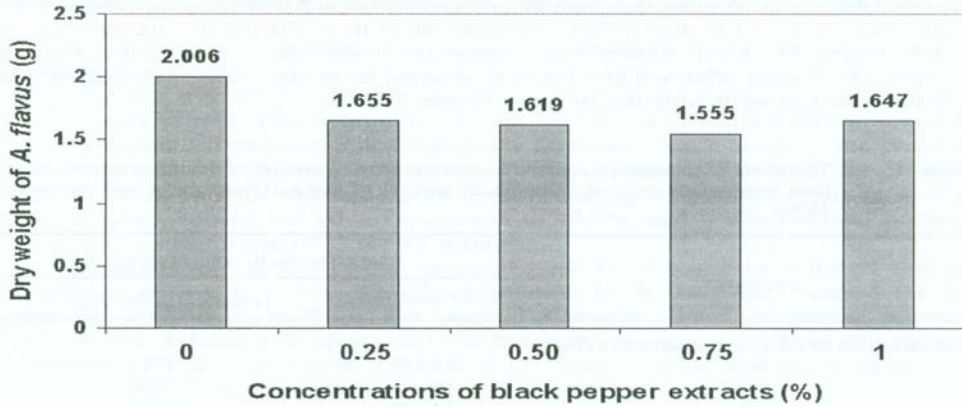


Figure 8. Dry weight of *A. flavus* on SMKY liquid medium containing different concentrations of black pepper extracts (Retnowati *et al.* 1998).

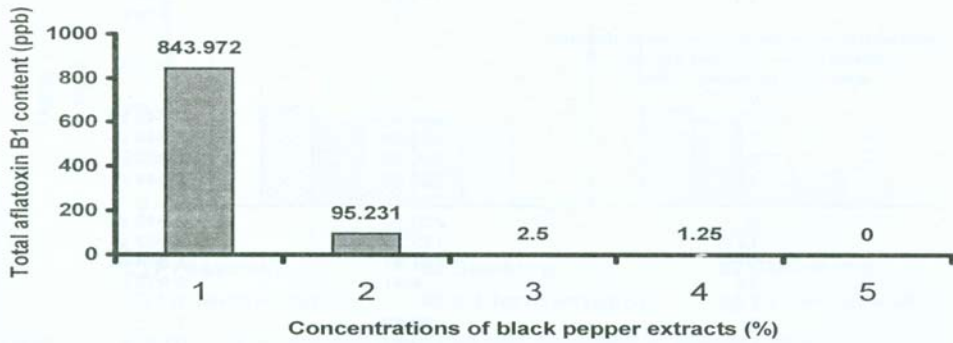


Figure 9. Total aflatoxin B₁ content produced by *A. flavus* on SMKY liquid medium containing different concentration of black pepper extracts (Retnowati *et al.* 1998).

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 B₁ content of maize (Dharmaputra and Ambarwati 1999).

Treatment	Total aflatoxin B ₁ content (ppb)	
	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
Interaction between ammonium hydroxide concentrations (%) and duration of ammonia treatment (hours)		
0		
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	8.41	0.922 o
1.0		
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 j
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. flavus*, 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 "black oncom", the presscake was fermented with *Rhizopus oligosporus*, while to make "red oncom" the presscake was fermented 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 B₁ 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.

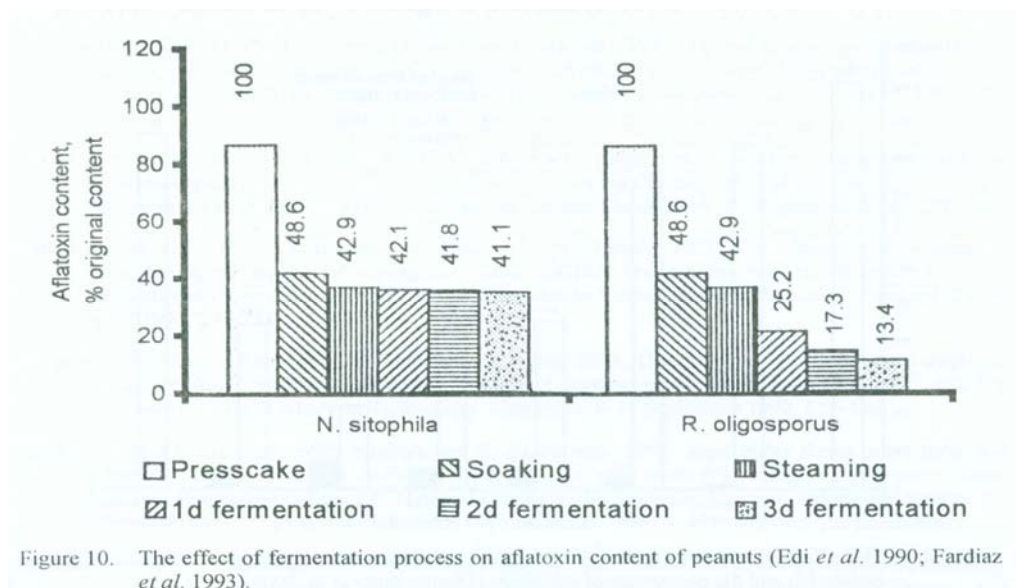


Figure 10. The effect of fermentation process on aflatoxin content of peanuts (Edi *et al.* 1990; Fardiaz *et al.* 1993).

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 g/m²/24 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 10₂, followed by non-toxicogenic *A. flavus* isolate 61₂ (61%), *A. tamaritii* (60%) and non-toxicogenic *A. flavus* isolate 36, (59%).

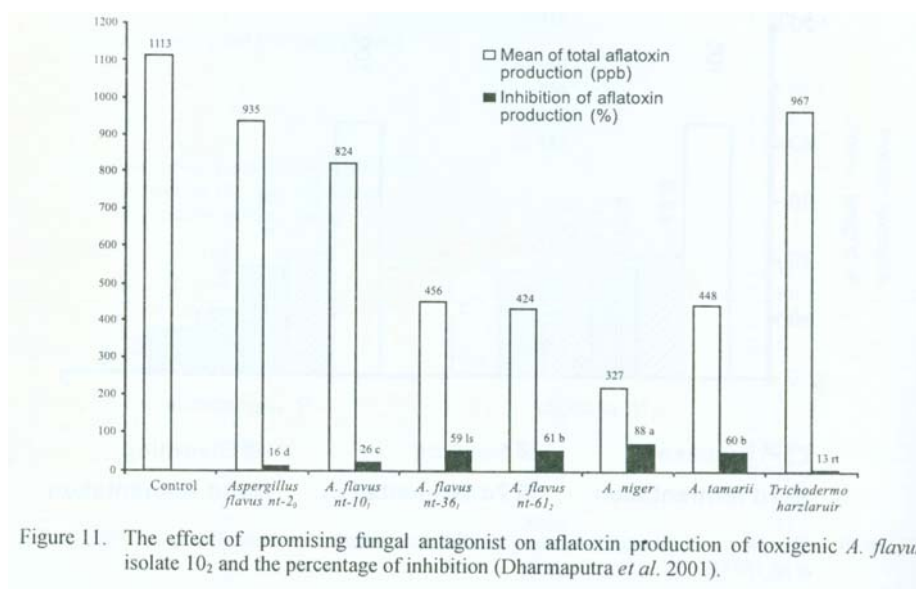


Figure 11. The effect of promising fungal antagonist on aflatoxin production of toxigenic *A. flavus* isolate 10₂ and the percentage of inhibition (Dharmaputra *et al.* 2001).

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.

REFERENCES

- Bulaong, S.S.P. and O.S. Dharmaputra. 2002. Fungal population, aflatoxin and free fatty acid contents of peanuts packed in different bag types. *BIOTROPIA* No. 19 : 1 - 25.
- Dharmaputra, O.S., H.S.S. Tjitrosomo, F. Aryani and M. Sidik. 1990. The effect of carbondioxide on storage fungi of maize. Proceedings of the 5th International Working Conference on Stored-product Protection, Bordeaux, France, 9-14 September 1990,291-301
- Dharmaputra, O.S., H.S.S. Tjitrosomo, H. Susilo and Sulaswati. 1991. *Aspergillus flavus* and aflatoxin of peanuts collected from three markets in Bogor, West Java, Indonesia. Proceedings of the 12th Asean Seminar on Grain Postharvest Technology, Surabaya, Indonesia, 29-31 August 1989, 110 -123.
- Dharmaputra, O.S., H.S.S. Tjitrosomo, T.S. Wardani and H. Halid. 1992. The effect of carbondioxide on some biological aspects of *Aspergillus flavus*. Proceedings of the 13th ASEAN Seminar on Grain Postharvest Technology, Bandar Seri Begawan, Brunei Darussalam, 4-7 September 1990, 280-293
- Dharmaputra, O.S., H.S.S. Tjitrosomo, M. Sidik and R.C. Umaly. 1991. The effects of phosphine on some biological aspects of *Aspergillus flavus*. ACIAR Proceedings No. 36. Proceedings of an International Conference on Fungi and Mycotoxins in Stored products, Bangkok, Thailand, 23-26 April 1991,244-248
- Dharmaputra, O.S., I. Retnowati, M. Sidik and H. Halid. 1993. The effects of phosphine and length of storage on fungi, aflatoxin and protein content of soybean meal. Proceedings of the 15th ASEAN Seminar on Grain Postharvest Technology, Singapore, 8-11 September 1992, 125-136
- Dharmaputra, O.S., I. Retnowati, Sunjaya and S. Ambarwati. 1995. *Aspergillus flavus* population and aflatoxin content in maize collected from farmers and traders in Lampung province, South Sumatra. Proceedings of the 12th National Congress and Scientific Seminar, Indonesian Society for Phytopathology, Yogyakarta, Indonesia, 6-8 September 1993. Vol. 1, 560-566. (In Indonesian).
- Dharmaputra, O.S., I. Retnowati, H.K. Purwadaria and M. Sidik. 1996a. Surveys on postharvest handling, *Aspergillus flavus* infection and aflatoxin contamination of maize collected from farmers and traders. Paper presented at the 17th ASEAN Technical Seminar on Grain Postharvest Technology, Lumut, Perak, Malaysia, 25-27 July 1995. ACIAR Technical Reports 37, 38-53.
- Dharmaputra, O.S., and A.S.R. Putri. 1996b. *Aspergillus flavus* population and aflatoxin content in maize, maize products and chicken feed. Paper presented at the National Microbiology Seminar and Annual Scientific Meeting, Malang, Indonesia, 12-13 November 1996. (In Indonesian).
- Dharmaputra, O.S., H. Susilo and M. Sidik. 1997. Population of storage fungi and aflatoxin content of soybean meal. Proceedings of the 14th National Congress and Scientific Seminar, Indonesian Society for Phytopathology, Palembang, Indonesia, Vol. 2,241-249. (In Indonesian).

- Dharmaputra, O.S., A.S.R. Putri and W. Setiawati. 1999. Fungal infection and possibility of aflatoxin contamination in black and white pepper. *Hayati* 6(3): 70-73. (In Indonesian with abstract in English).
- Dharmaputra, O.S. and S. Ambarwati. 1999. Detoxification effects of ammonium hydroxide on aflatoxin and total nitrogen content of maize. *Hayati* 6(2): 25-28
- Dharmaputra, O.S., I. Retnowati, M. Amad and S. Ambarwati. 2000. Airtight storage of maize: its effect on fungal infection and aflatoxin production. Proceedings of the 19th ASEAN and 1st APEC Seminar on Postharvest Technology, Ho Chi Minh City, Vietnam, 9-12 November 1999, 474-482
- Dharmaputra, O.S., A.S.R. Putri, I. Retnowati and S. Ambarwati. 2001. Antagonistic effect of soil mycobiota on aflatoxin-forming *Aspergillus flavus* *in vitro*. Paper presented at the 20th ASEAN and 2nd APEC Seminar on Postharvest Technology. Chiang Mai, Thailand, 11-14 September 2001.
- Dharmaputra, O.S., A.S.R. Putri and Lilieanny. 2002. Population of storage fungi and aflatoxin content in peanut products. Internal Report. SEAMEO BIOTROP. (In preparation).
- Edi, S. Fardiaz and D. Fardiaz. 1990. Production of aflatoxin by *Aspergillus flavus* and its destruction during fermentation of peanuts by *Neurospora sitophila*. *Buletin Penelitian Ilmu dan Teknologi Pangan* 1(1): 18-31. (In Indonesian).
- Fardiaz, S., S. Wulan and B. Setiyadi. 1993. Effect of fermentation by *Rhizopus oligosporus* on aflatoxin production by *Aspergillus flavus* in peanut presscake. Proceedings of the 5th ASEAN Food Conference, Jakarta, Indonesia, February 1992, 858-864
- Haryadi, Y. and E. Setiastuty. 1994. Characterisation of aflatoxins B₁, B₂, d and G₂ in groundnuts and groundnut products. Proceeding of the 6th International Working Conference on Stored-product Protection, Canberra, Australia, Vol. 2, 996-998.
- Maryam, R. 1994. Kontaminasi asam siklopiazonat dan aflatoksin pada jagung. Makalah dibawakan pada Kongres Nasional Perhimpunan Mikologi Kedokteran Manusia dan Hewan Indonesia I dan Tcmu Ilmiah. Bogor, 21-24 Juli 1994, 289-293
- Pitt, J.I. and A.D. Hocking. 1996. Current knowledge of fungi and mycotoxins associated with food commodities in Southeast Asia. *Mycotoxin Contamination in Grains*. ACIAR Technical Reports 37, 5-10
- Purwoko, H.M., B. Hald and J. Wolstrup. 1991. Aflatoxin content and number of fungi in poultry feedstuffs from Indonesia. *Letters in Applied Microbiology* 12: 212-215
- Putri, A.S.R., O.S. Dharmaputra, Sunjaya and M. Sidik. 1999. The effectiveness of phosphine to maintain the quality of maize packed in two different bag types. Proceedings of the 7th International Working Conference on Stored-Product Protection, Beijing, P.R. China, 14-19 October 1998, 274-279
- Retnowati, I., O.S. Dharmaputra, H. Susilo and H. Affandi. 1998. Inhibitory effects of black pepper and turmeric extracts on the growth and aflatoxin production of *Aspergillus flavus*. Proceeding of the National Microbiology Seminar and Annual Scientific Meeting, Bandar Lampung, Indonesia, 14-15 December 1998, 210-220. (In Indonesian with abstract in English)
- Ventikitasubramanian, T.A. 1977. Biosynthesis of aflatoxin and its control. In Rodricks, J.V., C.W. Hesseltine and M.A. Mehlman (eds.). *Mycotoxin in Human and Animal Health*. Pathotox Publishers Inc., part Forest South, Illinois, 83-98
- Zahari, P. and Tarmudji. 1995. Aflatoxikosis pada ternak itik Alabio di Kalimantan Selatan. Presiding Seminar Nasional Teknologi Veteriner untuk Meningkatkan Kesehatan Hewan dan Pengamanan Bahan Pangan Asal Ternak. Cisarua, Bogor, Indonesia, 22-24 Maret 1994, 408-411